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EphrinB2 expression in prostate adenocarcinoma: Implications for targeted therapy

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Abstract

Background: Prostate cancer is managed by surgery, androgen deprivation and cytotoxic chemotherapy. Targeted therapy is emerging as an important pillar in cancer therapeutics, however, efficacy in prostate cancer has been limited. Eph-ephrin is a novel pathway that is upregulated in prostate cancer and promotes the initiation and progression of cancer. The aim of this study was to determine the immunohistochemical expression of ephrinB2 in prostate adenocarcinoma.

Methods: A tissue microarray comprising of prostate adenocarcinoma of different grade groups was stained with a monoclonal anti-ephrinB2 antibody (Abcam, AB201512). The tumor and endothelial cells expressing the ephrinB2 positivity were noted. The statistical analysis was performed to determine the difference in expression based on grade groups and the TNM stage.

Results: EphrinB2 was expressed in 40 out of 72 cases (55.5 %) of prostate adenocarcinoma and was pre-dominantly negative in the normal prostatic tissue. There was no significant difference in the expression of ephrinB2 in various grade groups (p = 0.7) or stages (p = 0.6).

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Declaration of Competing Interest

David Quinn: Honoraria from Dendreon, Bayer, Astellas, Janssen, Sanofi, Clovis, AstraZeneca, Celgene, Mundipharma
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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.prp.2020.152967.

Conclusions: EphrinB2 is expressed in a significant number of prostate adenocarcinoma regardless of grade and stage. Hence, there is a potential to target this molecule in the low-grade tumors with localized disease as well as high grade, high volume tumors with metastatic disease.

Keywords

Prostate adenocarcinoma; EphrinB2; Targeted therapy; Immunohistochemistry; Eph-ephrin

1. Introduction

Eph receptors comprise the largest family of receptor tyrosine kinases (RTK), with 14 members divided into A and B classes, according to the sequence homology. Class A includes 9 members named EphA1−8 and, EphA10; while class B includes 5 members named EphB1−4 and, EphB6 [1]. Their ligands too are divided into classes A and B depending upon their binding to the class of receptor; with 5 and 3 members in class A and B, respectively. The EphA and EphB receptors have identical structures with extracellular, transmembrane and intracellular regions. Ligands for Eph RTKs or ephrins are cell membrane-bound proteins; EphrinsA are GPI-anchored surface proteins while ephrinsB are transmembrane proteins [2]. Because the ephrin ligands are cell-surface proteins, cell-cell contact is needed for receptor-ligand interaction. Eph-ephrin binding leads to bidirectional signaling with activation of the receptor called forward signaling while signaling through the ligand termed reverse signaling [3]. Ephs and ephrins have varying roles such as axon guidance, cell migration, and vascular development and maturation [1]. The deregulated expression of these proteins in adults plays an important role in neoangiogenesis, tumor progression, invasion, and metastasis in human cancers [3–5].

Although the Eph and ephrins are divided into two different classes, interclass binding of the receptors and ligands is also well known [4]. Accordingly, the ephrinB2 activates and is activated by several different EphB molecules; however, for the EphB4 receptor, it is the only activator [6]. The activation of EphB4 leads to tumor cell attachment and migration, while the reverse signaling of ephrinB2 leads to tumor angiogenesis [7]. Hence, evaluating and targeting the EphB4-ephrinB2 pathway remains one of the important therapeutic strategies. The expression of EphB4 has been studied in prostate cancer cell lines and clinical prostate specimens [8,9]. However, the studies on immunohistochemical expression of ephrinB2 in prostate cancer is largely restricted either to the cell lines or a limited number of clinical specimens [10,11]. Further, ephrinB2 overexpression in other solid tumors is associated with poor prognosis and response to therapy [12]. Studies have even validated the therapeutic potential of blocking ephrinB2 molecule [13–15]. Keeping the above facts in mind, we in this study evaluated the immunohistochemical expression of ephrinB2 in prostate adenocarcinoma.

2. Material and methods

2.1. Prostate tissue specimen

Prostate tissue microarrays (TMA) were obtained from a commercial supplier (US Biomax, Rockville, MD; TMA catalog number PR1921c). The TMA comprised of the specimen

from 96 patients consisting of 80 prostate adenocarcinomas, 8 tumor-adjacent tissues, and 8 normal prostate tissues, with duplicate cores per case. The tissue samples were formalin-fixed paraffin-embedded. Individual tissue cores were 1.0 mm in diameter and 5 μm in thickness. US Biomax supplied the following clinicopathologic characteristics for each case: age, diagnosis, TNM stage, and Gleason Score (GS). The TMA slide was stained for the hematoxylin/eosin (HE) stain and the slide was evaluated by two experienced pathologists. The GS assigned to each individual case by the company was confirmed, and the Grade Group (GG) was assigned based on the confirmed GS. The consensus was reached in all cases.

2.2. Antibody

Anti-ephrinB2, a monoclonal antibody produced in rabbit was purchased from Abcam plc. (San Francisco, CA; clone AB201512). We validated this antibody for use in immunohistochemistry (IHC). Isogenic CHO (Chinese hamster ovary) cell lines were prepared by stable expression of ephrinB2, ephrinB1, and ephrinB3. Wild type CHO does not express ephrinB2. Only CHO/ephrinB2 showed membrane staining with the antibody. Secondly, we used human normal tissue array. No expression was seen in normal tissues consistent with ephrinB2 being an embryonic protein.

2.3. Immunohistochemical staining

IHC was performed using the monoclonal antibody clone for ephrinB2 (AB201512, Abcam, San Francisco, CA). TMA slide was deparaffinized using xylene and rehydrated in graded alcohol. Antigen retrieval was accomplished by using citrate buffer (pH 6.5) and heat plate (100 °C). The primary antibody (ephrinB2) was used in 1:500 dilution. After several washes, slides were incubated with HRP polymer secondary antibodies and the antigen-antibody reaction was visualized using DAB chromogen. Slides were then counterstained with hematoxylin stain and IHC results were scored by two experienced pathologists. Immunoexpression of ephrinB2 was studied in tumor cells and endothelial cells of tumor blood vessels. The component stained (cytoplasm, membrane, nucleus), percentage of cells stained, and intensity of the staining (weak, 1+; moderate, 2+ and strong, 3+) were noted. Staining was defined as positive when at least 10 % (arbitrary cut-off) of tumor or endothelial cells displayed membrane expression of any intensity. Apical luminal staining within the tumor glands was considered positive. The case was considered positive when at least one of the duplicate cores showed tissue staining of defined criteria. The maximum score (for the intensity and percentage cells stained) of the 2 cores was considered for the statistical analysis. Divergences in staining interpretations were resolved by consensus.

2.4. Statistical analysis

For all statistical analysis, IBM SPSS statistics software version 24.0 was used. A chi-square test was used to find the correlation between the variables. Statistical significance was defined as $p < 0.05$.

3. Results

The GS assigned by the US Biomax was confirmed in all 80 tumor cases, however, 3 cases (2 assigned with GS 2 + 3 and 1 with GS 3 + 2) were excluded from the final analysis. Moreover, the IHC was not interpretable in 5 additional cases due to various reasons (cores washed off and folded section in 2 cases each, while tumor depletion in 1 case). Thus, 72 cases formed the final study sample, each with duplicate cores. The age of these patients ranged from 57 years to 97 years (median 70 years). Of the total 72 cases of prostate adenocarcinoma, the number of cases with GG 1 through 5 was 6, 14, 5, 22 and 25, respectively. EphrinB2 IHC was expressed in 40 out of 72 cases (55.5 %). Number of cases with positive ephrinB2 immunoexpression in grade groups 1 through 5 were 3/6 (50 %), 7/14 (50 %), 4/5 (80 %), 13/22 (59 %) and 13/25 (52 %), respectively (Table 1) (Fig. 1). Majority of the cases displayed moderate (60 %) to strong (32.5 %) immunoexpression, while weak intensity was noted in 7.5 % cases (Table 1) (Fig. 2). Expression limited to the tumor cells or the endothelial cells was seen in 28 (70 %) and 8 cases (20 %), respectively; while both the tumor cells and endothelial cells were positive in 4 cases (10 %) (Table 1) (Fig. 3). Chi-square test for the ephrinB2 expression in different grade groups did not show any significant statistical correlation ($p = 0.7$) (Table 1). There was no significant statistical correlation between the expression of ephrinB2 and TNM stage ($p = 0.6$) (Table 1). Also, there was no statistical correlation between the percent of cells stained and the GG or stage. Three out of 8 tumor-adjacent tissues showed endothelial expression of ephrinB2, while only 1 out of 8 normal prostate tissues showed endothelial positivity. None of these cases displayed the glandular positivity of ephrinB2 (Fig. 4).

4. Discussion

The Eph-ephrin receptors-ligands combinations (especially EphB4-ephrinB2) have been studied in many cancers, including that of the lung, breast, head neck, brain, ovarian, esophageal, colorectal, and Kaposi sarcoma; hence these molecules are attractive targets for cancer therapy [4,16–19]. Eph-ephrin interactions can be targeted by numerous therapeutic agents such as soluble Eph and ephrin exodomain fusion proteins, monoclonal antibodies, peptide vaccines, Eph kinase domain inhibitors, small interfering RNAs, antisense oligodeoxynucleotides, or dendritic cell-based tumor vaccines [3–5,8,20]. Studies have shown that EphB4 levels are increased in prostate cancer compared to benign epithelial cells, and its expression correlates with the invasiveness and metastatic potential of the tumor cells [8–10,21]. However, the evaluation of the immunohistochemical expression of ephrinB2 in prostate cancer is not thoroughly assessed to date [10,11]. This is also important in the light of the recent study that has not only highlighted the poor prognosis and response to therapy associated with overexpression of ephrinB2 in solid tumors but has also shown the therapeutic potential of blocking ephrinB2 ligand [12]. Hence, in this study, we aimed at evaluating the immunohistochemical expression of this imminent potential target (ephrinB2) in prostate adenocarcinoma. We found that there was no statistically significant difference in the expression of ephrinB2 in all the GG tumors ($p = 0.7$) and stages ($p = 0.6$). These findings suggest that ephrinB2 expression is not related to the grade of the tumor. Therefore, therapy targeting ephrinB2 can be explored irrespective of the grade or

stage of the tumor; wherein the patients with the localized disease might be benefited from non-surgical measures while it will also have a role to play in a metastatic setup.

During development, EphB4 and ephrinB2 are characteristically expressed in the veins and the arteries respectively [1]. This expression pattern enables vascular remodeling and venous-arterial segregation [22,23]. Ozgur et al. found that prostate cancer as compared to normal tissue expresses a higher intensity of ephrinB2 in the arteries [11]. Our study further confirms this finding as there was a significant difference between the vascular expression of ephrinB2 in the tumor (n = 12/72) and tumor-adjacent tissue (n = 3/8) as against the normal prostate tissue (n = 1/8). Further, the EphB4 on the tumor cells stimulates the ephrinB2 positive vascular cells and promotes the formation of blood vessels which in turn increases tumor growth [2]. The role played by EphB4-ephrinB2 in tumor angiogenesis and the ability of soluble monomeric derivative of the extracellular domain of EphB4 (sEphB4) to modulate this process is well established [6]. Considering the above facts, the test was interpreted positive in 8 cases wherein only blood vessels were stained by immunohistochemistry. Nonetheless, the expression of ephrinB2 is still seen in a significant number of cases (n = 32/72, 44 %), even if the ‘vessel only’ positivity is neglected. It is also important to note that in contrast to VEGF overexpressed tumors, ephrinB2 overexpressed tumors have more efficient tumor vasculature that may facilitate the delivery of antineoplastic drugs to the cancer tissue [2]. Further, inhibiting the EphB4-ephrinB2 pathway hampers tumor angiogenesis leading to hypoxia and induction of VEGF expression [18]. Simultaneously targeting the VEGF and EphB4-ephrinB2 is another potentially effective therapy [24]. The above findings thus reiterate the importance of interpreting the ephrinB2 expression in the blood vessels.

The therapeutic agents studied for targeting EphB4-ephrinB2, in particular, include sEphB4, EphB4 small interfering RNA, EphB4 antisense oligodeoxynucleotides, and EphB4 kinase domain inhibitors [6–8,18,25–27]. sEphB4 binds to ephrinB2 and blocks activation of both EphB4 receptor and ephrinB2 ligand and thus negatively affects angiogenesis and inhibits tumor growth [6]. This ephrinB2 decoy receptor has not only shown a tumor growth delay and survival improvement in head-neck squamous cell carcinoma (HNSCC) but has also shown to be a radiosensitizer in this tumor [12,13]. In HNSCC, its use has shown an enhanced response to cetuximab-radiotherapy combination treatment and it appears to be an effective alternative to anti-PDL1 to be used with radiotherapy for inducing anti-tumor immune response [14,15]. Targeting the EphB4-ephrinB2 pathway in prostate cancer tumor cell lines and murine tumor xenograft models has shown anti-tumor activity and it will be interesting to see the activity of sEphB4 in this tumor in a clinical setup [8]. There are many undergoing trials with this agent with at least two dealing with prostate cancer (NCT04033432, NCT02767921) [28]. The safety of this drug has been established in many recent clinical trials (NCT01642342, NCT02717156, and NCT02767921) [15].

This study does have certain limitations. While all tumor cores included in the analysis contained a tumor, it is possible that some of the cases with negative expression have other regions of the tumor with positive expression (due to tumor heterogeneity). Hence, the true expression may be somewhat higher than the actual frequency reported in this study. The interpreted estimate of ephrinB2 expression in the blood vessels might be lower in the

regions of high-grade tumors (closely packed glands) with high ephrinB2 expression. The relative numbers of GG1 and GG3 tumors in this study are less and hence the findings in this study need to be further validated by a future prospective study including a larger cohort of cases with survival data. The cut-off for IHC positivity was arbitrarily taken as 10 % which can be further refined by using several available tools in a larger cohort [29]. Although we used both, commercial antibody and TMA, the expression of ephrinB2 in a significant number of prostate adenocarcinoma is irrefutably emphasized by this study. Nonetheless, this study sets a platform from where the future prospective studies can be taken off.

5. Conclusion

In addition to EphB4, cognate high-affinity ligand ephrinB2 is also induced in prostate adenocarcinoma and prostate tumor vessels; thus being a potential target for therapy. It is expressed in the majority of clinical prostate adenocarcinoma specimens and in all the GG and tumor stages. Thus potentials of targeting this pathway in any GG tumors with any stage should be explored.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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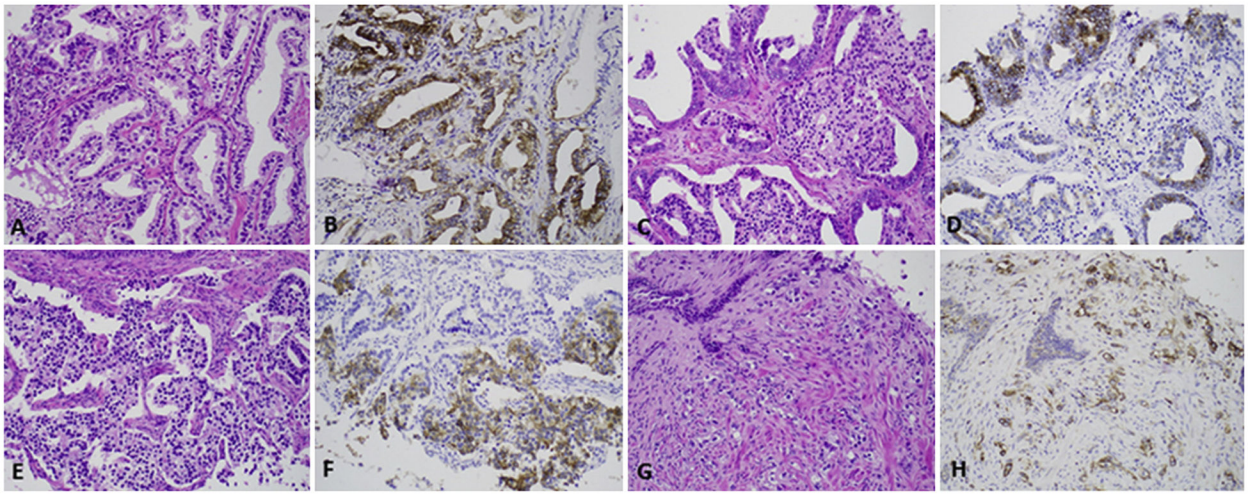

Fig. 1.

Expression of ephrinB2 in different Grade Group tumors (H&E, IHC 200x): **A-B)** Grade Group 1 tumor (GS 3 + 3 = 6) showing strong membrane expression of ephrinB2, **C-D)** Grade Group 3 tumor (GS 4 + 3 = 7) showing focal strong immunoreactivity for ephrinB2, **E-F)** Grade Group 4 tumor (GS 4 + 4 = 8) showing moderate expression of ephrinB2, **G-H)** Grade Group 5 tumor (GS 5 + 5 = 10) showing solitary isolated tumor cells that are positive for ephrinB2 IHC.

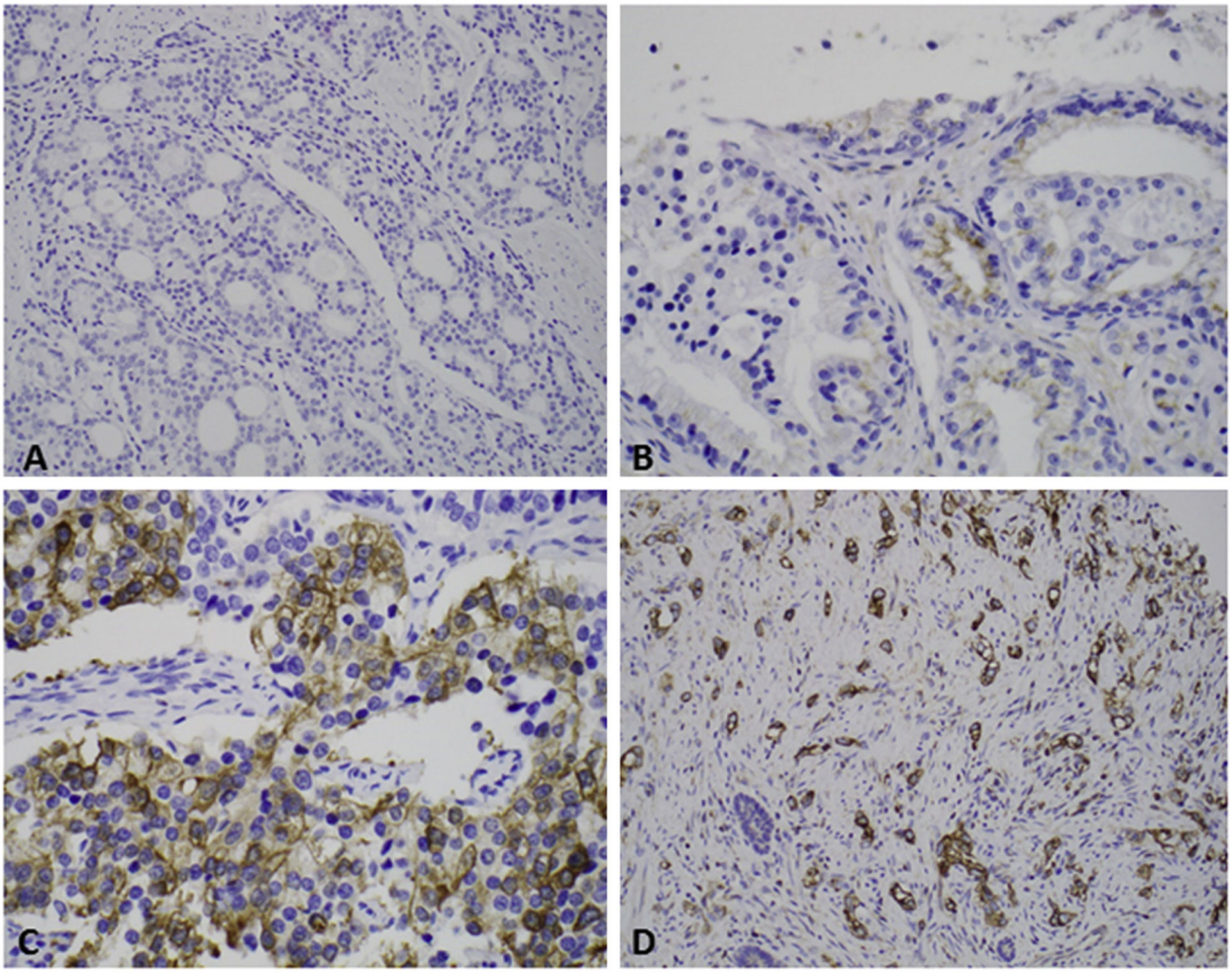

Fig. 2.

EphrinB2 IHC intensity of staining: **A)** Prostate adenocarcinoma showing the absence of staining for ephrinB2 (200x). **B-D)** Expression pattern displayed varied intensity of staining; weak 1+ **(B, 400x)**, moderate 2+ **(C, 400x)** and strong 3+ **(D, 200x)**.

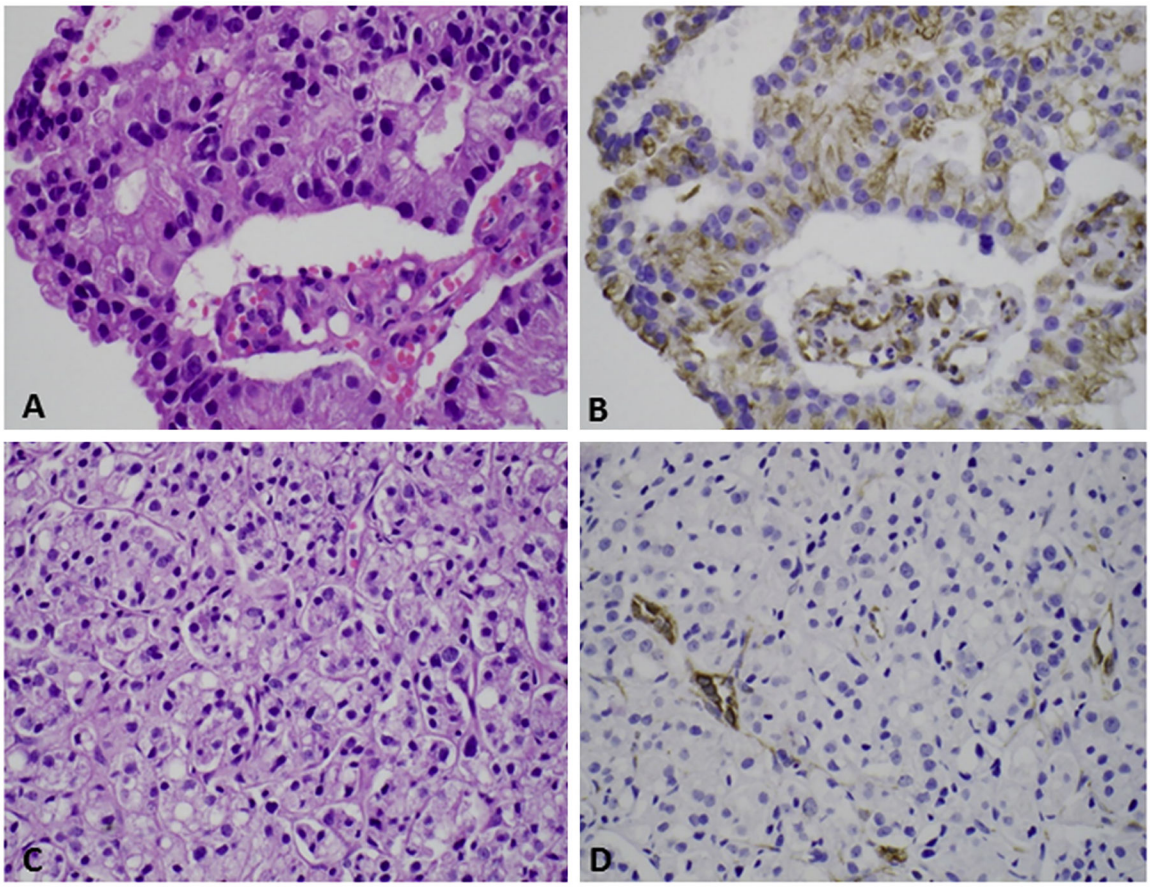

Fig. 3.

Expression in endothelial cells (H&E, IHC 400x): **A-B)** Endothelial cells showing the presence of ephrinB2 expression in vessels within the tumor, **C-D)** Expression of ephrinB2 in the endothelial cells alone in the regions of tumor negative for ephrinB2.

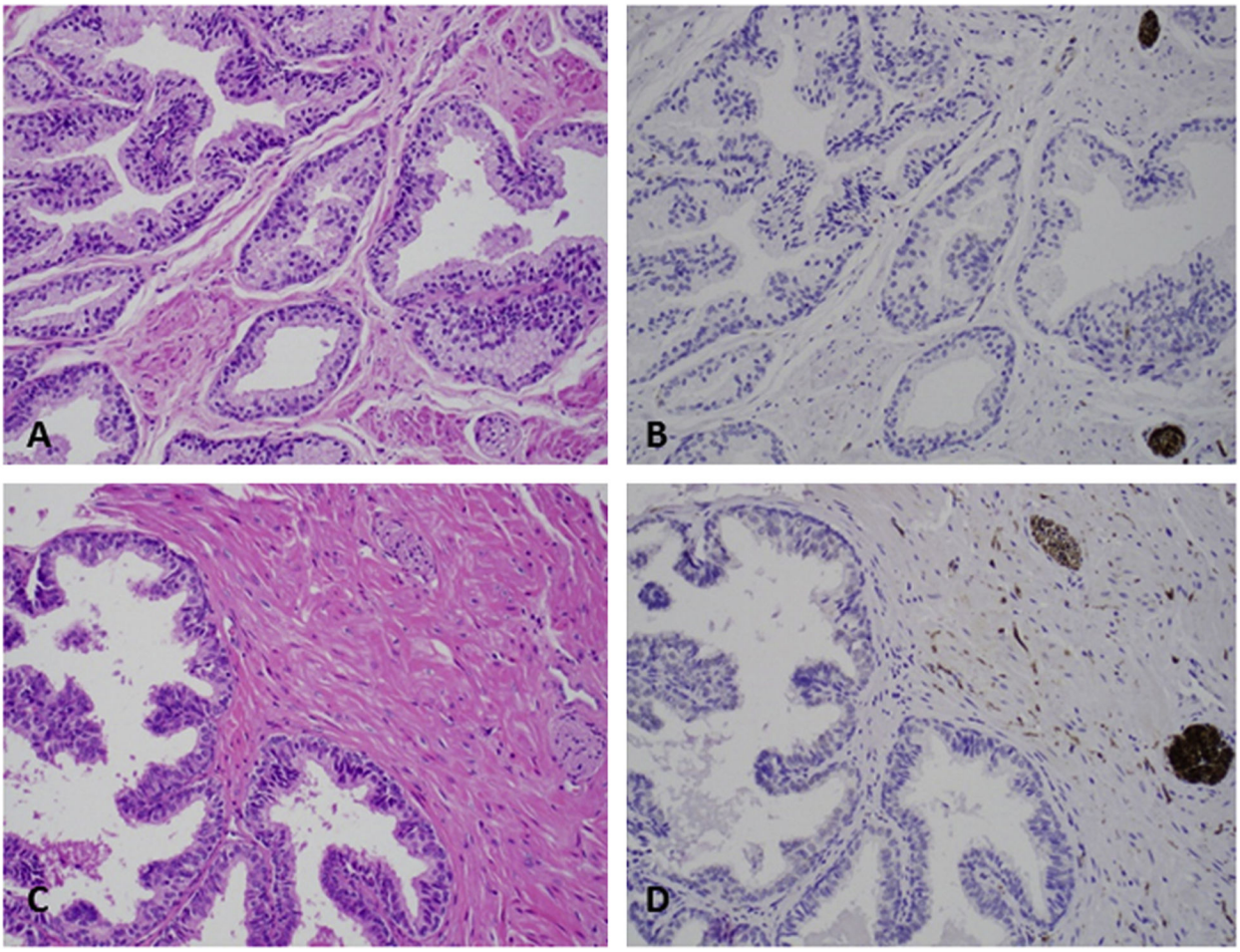

Fig. 4.

Adjacent normal tissue and normal prostatic tissue (H&E, IHC 200x): Cores from the tumor-adjacent tissue **(A-B)** and normal prostate **(C-D)** did not display any glandular positivity. The nerve fibers are stained positive (B, D) which serve as an internal control.

Table 1

Distribution of Ephrin B2 immunohistochemical expression according to different variables.

		Total Cases	Positive cases (No.)	Positive Cases (%)	p-value
Ephrin B2 intensity of staining in prostate adenocarcinoma	**Weak (1+)**		3	7.5 %	
	Moderate (2+)		24	60 %	
	Strong (3+)		13	32.5 %	
	Total	**72**	**40**	55.5 %	
Tissue component in prostate adenocarcinoma expressing ephrinB2	**Tumor Cells**		28	70 %	
	Endothelial Cells		8	20 %	
	Endothelial + Tumor Cells		4	10 %	
	Total	**72**	**40**	55.5 %	
EphrinB2 expression in different Grade Groups of prostate adenocarcinoma	**Grade Group 1** (Gleason Score 3 + 3 = 6)	6	3	50 %	**0.7**
	Grade Group 2 (Gleason Score 3 + 4 = 7)	14	7	50 %	
	Grade Group 3 (Gleason Score 4 + 3 = 7)	5	4	80 %	
	Grade Group 4 (Gleason Score 8)	22	13	59 %	
	Grade Group 5 (Gleason Score 9–10)	25	13	52 %	
	Total	**72**	**40**	55.5 %	
EphrinB2 expression in different stages of prostate adenocarcinoma	**Stage I**	3	1	33 %	**0.6**
	Stage IIA	10	7	70 %	
	Stage IIB	20	11	55 %	
	Stage III	16	7	44 %	
	Stage IV	5	3	60 %	
	Total	**54**	**29**	**54 %**	