Expression of Vascular Endothelial Growth Factor in Taiwanese Benign and Malignant Prostate Tissues

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Background: The expression of vascular endothelium growth factor (VEGF) has been correlated to the grading and stage of prostate cancers. However, data regarding Taiwanese prostate cancer patients are lacking. The aim of the present study was to examine VEGF expression in our radical prostatectomy specimens.

Methods: Fifty-one radical prostatectomy specimens with prostate cancer (15 stage pT2N0, 25 pT3N0, 11 pT2-4 N1) were stained using goat anti-human VEGF polyclonal antibody (AB-293NA; R&D Systems Inc., Minneapolis, MN, USA). The VEGF expression in malignant and nonmalignant prostate tissues was compared. The correlations of VEGF immunoreactivity with Gleason scores and pathologic stages were examined. Mann–Whitney *U* test was used for comparison of preoperative prostate-specific antigen levels between patients with and without VEGF expression.

Results: Positive VEGF staining was observed in 80.4% of malignant epithelia, 39.2% of peritumoral stroma, 68.6% of benign hyperplastic glands, and 25.5% of adjacent stroma. There was no difference in VEGF expression between malignant and nonmalignant areas. Advanced disease had significantly higher frequency of stroma but not epithelium VEGF staining as compared to organ-confined disease ($p = 0.002$ and $p = 0.412$, respectively). The Gleason 7 and higher tumors had significantly higher frequency of VEGF staining in stroma but not glandular epithelium ($p = 0.041$ and $p = 0.353$, respectively). Tumors with positive epithelium VEGF staining had significantly higher PSA levels (21.3 ± 18.1 *vs.* 10.8 ± 6.8 ng/mL; $p = 0.013$).

Conclusion: There was no difference in VEGF immunoreactivity between malignant and benign prostatic epithelium in Taiwanese. High Gleason grade tumors and advanced disease had significantly higher frequency of VEGF expression in stroma but not glandular epithelium. Tumors with positive epithelium VEGF staining had significantly higher PSA levels. [*J Chin Med Assoc* 2007;70(9):380–384]

Key Words: angiogenesis factor, prostatic neoplasm, vascular endothelial growth factor

Introduction

Neoangiogenesis is critical for a solid tumor to grow, to progress and eventually to disseminate.[1] Vascular endothelium growth factor (VEGF) is a 45-kDa secretary glycoprotein responsible for endothelial cell differentiation, migration, proliferation, tubular formation and vessel assembly.[2–4] It has been proven to be an important promoter of tumor neovascularity.[5] Positive associations between tumor VEGF expression and aggressiveness have been demonstrated in colon,[6] breast[7] and gastric cancers.[8]

Published data regarding VEGF expression and distribution in benign and malignant prostate tissue is conflicting. Previous studies have found prominent VEGF expression in malignant glands and adjacent stroma but only weak staining in basal cells of benign hyperplastic glands.[9,10] Walsh et al reported greatest VEGF staining in the stroma of benign prostatic hyperplasia (BPH).[11] In the study of Jackson et al, widespread

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distribution of VEGF in prostate cancers and BPH specimens was demonstrated.[12] Joseph and Isaacs illustrated that androgen may regulate prostate cancer growth through upregulation of VEGF expression.[13] A recent study from China found overexpression of VEGF in malignant epithelium cells as compared with adjacent benign epithelium.[14]

We believe the discrepancies might at least in part result from differences in specimens used for study or the amount of VEGF sequestrated during that particular time frame. The aim of the present study was to compare the levels of VEGF expression in benign hyperplastic and malignant prostate tissue using radical prostatectomy specimens.

Methods

From the records of our department of pathology, we identified 51 radical prostatectomy specimens with prostate cancer (15 stage pT2N0, 25 pT3N0, 11 pT2-4N1). A representative section containing the largest tumor area was used for immunohistochemical study. Five age-matched patients who had undergone radical cystoprostatectomy for bladder cancer were selected for comparison. The protocol was approved by the institutional review board of Kaohsiung Veterans General Hospital.

Four-μm-thick sections from corresponding archival blocks were deparaffinized and rehydrated with xylene, 99% and 95% ethanol. Antigen retrieval was performed by soaking slides in a 10 mmol/L citrate buffer, pH 6, and heating them in a 90°C water bath for 20 minutes. Endogenous peroxidase activity was blocked by immersing slides in 3% H_2O_2 for 5 minutes. After washing with Tris-buffer, slides were incubated with goat anti-human VEGF polyclonal antibody (AB-293NA; R&D Systems Inc., Minneapolis, MN, USA) diluted 1:100 overnight at 4°C in a moist chamber. Slides were then washed twice in Tris-buffer over 10 minutes in total, incubated for 1 hour in biotinylated rabbit anti-goat antibody (DakoCytomation, Denmark) diluted 1:100 at room temperature, followed by 2 washes in Tris-buffer. Immunoreactivity was detected by the ChemMate™ DAKO EnVision™ detection kit (DakoCytomation) and counterstained with hematoxylin.

Two independent observers (JSW and TTW) examined all sections while blinded to the clinical data. The intensity of VEGF immunostaining was scored semiquantitatively as negative (−), weak (+), moderate (++) or strong (+++). A section was recorded as positive when > 25% of the examined area stained moderate to strong positive.[11] The glandular and stromal portions of prostate were scored similarly.

The correlations of VEGF expression with Gleason scores and pathologic stages were evaluated by Pearson's χ^2 test. The Mann–Whitney *U* test was used for comparison of preoperative serum prostate-specific antigen (PSA) levels between patients with and without VEGF expression. Pearson's χ^2 test was used to compare epithelium and stromal staining between malignant and nonmalignant prostate tissues. Analyses were performed using SPSS version 12.0 (SPSS Inc., Chicago, IL, USA), and *p* values of less than 0.05 were considered statistically significant.

Results

The median age of prostate cancer patients at operation was 69 years (range, 56–78 years). Preoperative PSA levels ranged from 4.2 to 94.1 ng/mL (median, 11.5 ng/mL). Nineteen (37.3%) tumors were Gleason 5 and 6, 32 (62.7%) were Gleason 7 and higher. The age of bladder cancer patients ranged from 68 to 74 years (median, 70 years).

VEGF staining was mainly in the cytoplasm. The distribution was heterogeneous, and intensity of immunoreactivity varied widely. Forty-one tumors (80.4%) had VEGF expression in malignant glandular epithelium. Positive VEGF staining was also observed in peritumoral stroma in 20 cases (39.2%). The intensity of VEGF staining was weaker in stroma compared to glandular epithelium (Figure 1). In benign hyperplastic glands, 35 (68.6%) glandular epithelia and 13 (25.5%) stroma stained positive for VEGF (Figure 2). There was no difference in VEGF expression between malignant and benign hyperplastic areas (Table 1). Advanced disease had significantly higher frequency of VEGF staining in peritumoral stroma compared to organ-confined disease ($p = 0.002$) (Table 2). There was no difference in epithelium VEGF expression between organ-confined and advanced disease ($p = 0.412$) (Table 2). Gleason 7 and higher tumors had significantly higher frequency of VEGF staining in stroma but not glandular epithelium ($p = 0.041$ and $p = 0.353$, respectively) (Table 3). Tumors with positive staining in glandular epithelium had a trend of higher PSA levels (21.3 ± 18.1 *vs.* 10.8 ± 6.8 ng/mL; $p = 0.013$, Mann–Whitney *U* test).

In all specimens obtained from bladder cancer patients, focally distributed VEGF staining with moderate to strong intensity was observed in hyperplastic glands. Only 1 out of 5 cases (20%) had weak and scanty VEGF expression in stroma.

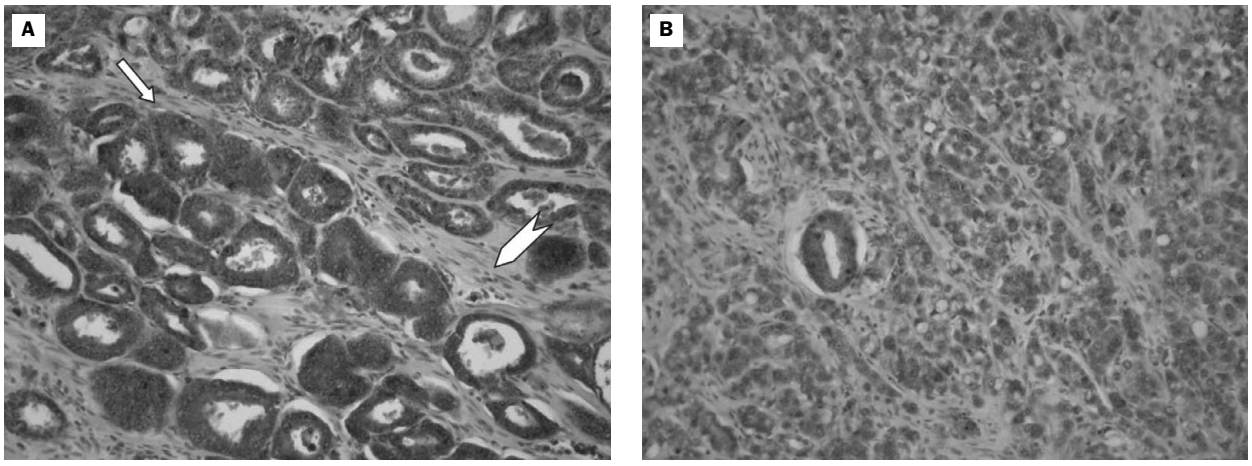

Figure 1. Vascular endothelium growth factor immunostaining in prostate cancer tissue: (A) strong positive stain in Gleason 3 tumor epithelium (arrow), but not in stroma (arrowhead); (B) diffuse cytoplasmic stain in Gleason 4 tumor and also peritumoral stroma (200×).

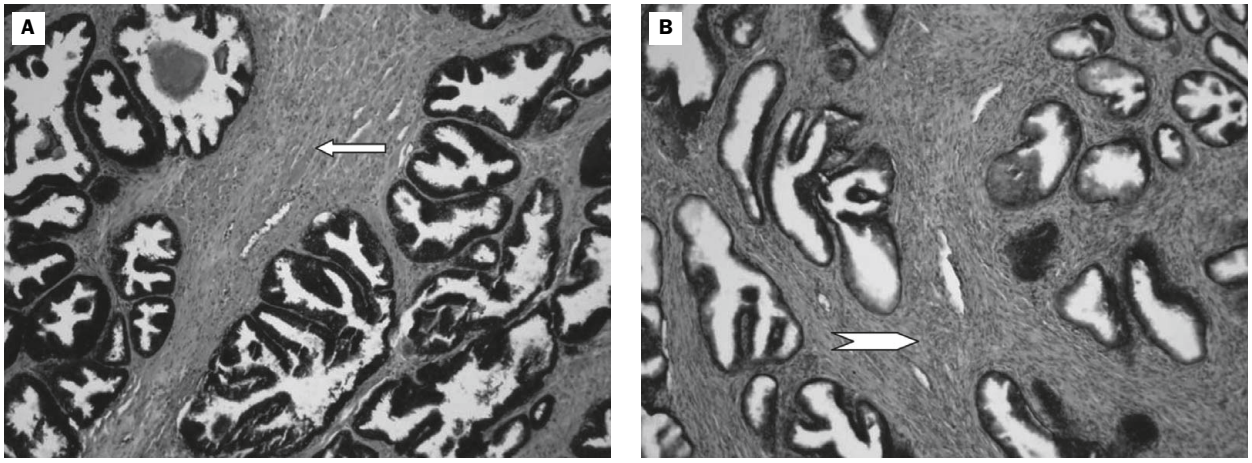

Figure 2. Vascular endothelium growth factor immunostaining in benign prostatic hyperplasia tissue: (A) strong positive stain in epithelium but not stroma (arrow); (B) positive stain in epithelium and weaker stain in stroma (arrowhead) (200×).

Table 1. Vascular endothelium growth factor (VEGF) expression in malignant and benign prostatic tissue (radical prostatectomy specimens only)*

	VEGF(+)	VEGF(–)	$p^{\dagger}$
Epithelium			0.173
Malignant	41 (80.4)	10 (9.6)	
Benign	35 (68.6)	13 (31.4)	
Stroma			0.138
Malignant	20 (39.2)	31 (60.8)	
Benign	13 (25.5)	38 (74.5)	

**Data are presented as n (%); †Pearson's χ^2 test.*

Table 2. Association of tumor vascular endothelium growth factor (VEGF) expression with pathologic stage*

	Epithelium		Stroma	
	VEGF(+)	VEGF(–)	VEGF(+)	VEGF(–)
Stage				
T2N0	11 (73.3)	4 (26.7)	1 (6.7)	14 (93.3)
T3 & N1	30 (83.3)	6 (16.7)	19 (52.8)	17 (47.2)
$p^{\dagger}$	0.412		0.002	

**Data are presented as n (%); †Pearson's χ^2 test.*

Discussion

Recent studies have shown that in addition to its well known angiogenic effect, VEGF may have a direct role in regulating tumor cell growth.[10,12,14] The VEGF signal transduction is carried out by way of 2 membrane receptors: fms-like tyrosine kinase 1 (FLT-1) and fetal liver kinase 1 (FLK-1).[15–18] Ferrer et al found expression of FLT-1 and FLK-1 in both prostate cancer and BPH specimens.[15] Moreover, co-localization of VEGF

Table 3. Association of tumor vascular endothelium growth factor (VEGF) expression with Gleason score

	Epithelium		Stroma	
	VEGF(+)	VEGF(–)	VEGF(+)	VEGF(–)
Gleason score				
5 & 6	14 (73.7)	5 (26.3)	4 (21.1)	15 (78.9)
7–9	27 (54.4)	5 (15.6)	16 (50.0)	16 (50.0)
$p^{\dagger}$	0.353		0.041	

**Data are presented as n (%); †Pearson's χ^2 test.*

and FLT-1 in prostate tumor cells further supports the hypothesis that VEGF exerts an autocrine regulation on prostate cell growth.[12,15]

Most previous studies have observed higher expression of VEGF in cancer epithelium as compared to nonmalignant glands.[9,10,14,19] However, inconsistent with the findings of Wash et al,[11] we found no difference in VEGF staining between malignant and benign prostatic epithelium. In both malignant and benign specimens we examined, positively stained glands were focally distributed and interspersed among negatively stained areas. The intensity of VEGF immunoreactivity was heterogeneous, ranging from weak to strong positive in single sections examined. Since both FLT-1 and FLK-1 receptors expressed consistently in cancer and BPH specimens, we believed that the VEGF staining might just represent the amount of VEGF produced or sequestrated in that area during a particular time frame. VEGF expression might not be related to the malignancy but reflect the ongoing autocrine regulation of tumor growth. This hypothesis was further supported by the findings of West et al that increased tumor epithelium VEGF immunoreactivity significantly correlated with higher serum PSA level.[9] In the present study, tumors with positive staining in glandular epithelium had significantly higher preoperative serum PSA level.

Correlation between VEGF expression and high Gleason score tumors have been reported by some investigators[20,21] but not by others,[12,14,19] including us. Ferrer et al even demonstrated more intense VEGF staining in well-differentiated tumors.[22] Expression of FLT-1 receptor was consistently found in both BPH and prostate cancer epithelium.[15] FLK-1 receptors were present in prostatic intraepithelial neoplasia and low-grade tumors but only little or none in poorly-differentiated tumors.[15] In hyperplastic glands, FLK-1 was localized mainly in the basal cell layer.[15] VEGF expression might not only be influenced by the amount of VEGF produced but also by the level of receptor existing on cell surface. Poorly differentiated tumors might have escaped from the autocrine regulation of VEGF.

Consistent expression of FLT-1 receptor has been found in fibromuscular stroma and vascular endothelial cells of prostate cancers.[15] The VEGF produced by stromal cells might be stimulated by some tumor-derived factors in a paracrine manner.[9,12] Expression of VEGF in stroma may thus indicate that a process of neoangiogenesis is propagating. Stroma VEGF immunoreactivity has been correlated to tumor stage and prognosis in the study of West et al.[9] In the present study, we observed significant association of peritumoral stroma VEGF immunoreactivity with pathologic stage ($p=0.002$) and high-grade tumor ($p=0.041$). VEGF expression in benign stroma might reflect highly vascularized or proliferating stroma.[12,14]

Our study is unique in using only radical prostatectomy specimens for assessing VEGF expression. Besides the peritumoral benign glands, pure BPH lesions in radical cystoprostatectomy specimens were also examined for comparison. In contrast, the samples that Yang et al examined included not only radical prostatectomy but also transurethral resection and needle-biopsy specimens.[14] That might explain why the observation in Chinese patients cannot be confirmed by the present study.

In conclusion, the present study found no difference in VEGF expression between malignant and benign prostatic epithelium in Taiwanese patients. Overexpression of VEGF in peritumoral stroma was associated with high Gleason scores and advanced stage, which again might indicate poorer prognosis. Further study is necessary to examine the association between VEGF expression and cell proliferation index.

Acknowledgments

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