Plasma levels and expression of vascular endothelial growth factor-A in human localized prostate cancer

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Abstract. Although the impact of vascular endothelial growth factor (VEGF) is clearly established in advanced prostate cancer (PCa), its role in localized PCa remains to be determined. The aim of our study was to analyse the plasma levels of VEGF-A and the expression of VEGF-A in prostatic tissue in a population of patients with localized PCa. We measured the preoperative plasma levels of VEGF-A in 100 patients undergoing radical prostatectomy (RP) for clinically-localized PCa. After intervention, we determined the expression of VEGF-A in all RP specimens using immunohistochemistry. We found no association between plasma levels of VEGF-A and the established prognostic factors of PCa. Moreover, there was no association between plasma levels of VEGF-A and the expression of VEGF-A in prostatic tissue. On the contrary, there was a strong correlation between the expression of VEGF-A in PCa tissue and the Gleason score of cancer: the expression of VEGF-A was significantly higher in patients with a high Gleason score on RP specimen (p=0.01). Our results suggest that the expression of VEGF may have a prognostic impact in clinically-localized PCa.

Introduction

Vascular endothelial growth factor (VEGF) is a glycoprotein acting as a specific endothelial mitogen. The VEGF gene family encodes five polypeptide growth factors: VEGF-A, -B, -C, -D and -E (1). VEGF-A can induce vascularization around growing tumor cells, and is overexpressed in prostatic tumors such as benign prostatic hyperplasia and prostate cancer (PCa) (2). It has been suggested that VEGF expression may be regulated by some tumor suppressor genes, including the PTEN (phosphatase, tensin homologue) gene on chromosome 10q23 (3). Several investigators found a correlation between circulating levels of VEGF-A and the aggressiveness of PCa, but others reached opposite conclusions (4-6). Moreover, most published studies have analysed the impact of VEGF-A in advanced PCa. The impact of VEGF-A in localized PCa remains therefore to be determined.

In the current study we measured the preoperative plasma levels of VEGF-A in 100 consecutive patients undergoing RP for localized PCa. We also examined the VEGF-A expression in the RP specimens, and analysed the associations between pre-RP VEGF plasma levels, VEGF expression in PCa tissue and established prognostic factors of PCa.

Materials and methods

Patient selection. One hundred consecutive patients undergoing RP for clinically-localized PCa at our institution were prospectively enrolled between June and November 2005. None had received hormone therapy, radiation therapy or chemotherapy before intervention. All patients had undergone pelvic CT scan, endorectal magnetic resonance imaging (MRI) and bone scintigraphy before RP. None had clinical or radiological evidence of lymph node or bone metastases. A bilateral pelvic lymphadenectomy was performed at the time of RP. Clinical and pathological stages of PCa were established according to the 2002 TNM classification.

Assessment of biomarkers (serum PSA and VEGF). Blood was taken from all patients the day before RP and was collected into two 7-ml glass tubes. One tube was used to measure the different forms of PSA. Total PSA, free PSA and complexed PSA were measured in serum using the Bayer Immuno 1 PSA assay. The other tube was used to measure plasma levels of VEGF-A. VEGF-A was measured using an enzyme-linked immunosorbent assay (ELISA). Blood was spun at 2500 g for 10 min; then, the platelet-poor plasma was removed and placed in a 5-ml tube, stored at -80˚C and thawed

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immediately before testing. VEGF-A levels were determined in duplicate with 100 μl of each sample. According to their amino acid content, 5 isoforms of VEGF-A have been identified, namely VEGF 121, VEGF 145, VEGF 165, VEGF 189 and VEGF 206. The immunoassay that we used (Bender MedSystems, Vienna, Austria) is designed to measure all these isoforms.

Tissue microarrays. Slides from the 100 RP specimens were reviewed and mapped. Tissue microarrays were built using a manual tissue arrayer (Beecher Instruments, Alphelys). Areas representative of the tumor with the highest Gleason score were circled. Duplicate 0.6 mm cores were obtained from the circled areas of tumor and transferred to a recipient paraffin block. Controls were obtained from non-malignant prostatic tissue. The tissue array set consisted of 8 blocks, and included the coordinates of each core and case of origin.

Immunohistochemistry. Immunochemical staining was performed on the section mounted on poly-L-Lysine coated glass slides. Before incubation with primary antibodies, deparaffinized and rehydrated sections were incubated with avidin/biotin blocker (Vector Laboratories, Burlingame, USA) and Fc receptor blocked by human serum (5%). Antigen retrieval was accomplished by heating the slides at 97˚C in a 0.01-M citrate buffer (pH 6.0) for 15 min. An anti-VEGF A-20 Mab (clone sc-152; Santa Cruz Biotechnology, CA, USA) was used at the dilution of 1:200 for 1 h. After rinsing in PBS, the biotinylated secondary antibody was applied for 30 min. To visualize the reaction, sections were incubated with an AEC substrate chromogen (Dakocytomation, Copenhagen, Denmark) for 15 to 20 min at room temperature. Slides were mounted with Glycergel (Dakocytomation) mounting medium and evaluated under a conventional light microscope. Breast carcinoma tissues were used as positive controls.

Evaluation of immunostaining. A semi-quantitative scoring system was used for the evaluation of immunostaining. Two pathologists (CB, PC) blinded to clinical data independently scored the slides. The intensity of staining was scored as 0, no detectable signal; 1, weak staining; 2, moderate staining; and 3, strong staining. The percentage of positive cells was scored as 0, no positive cells; 1, <1/3 positive cells; 2, 1/3-2/3 positive cells; and 3, >2/3 positive cells. Because of the duplicate nature of the arrays, two values were obtained for each patient. The highest intensity value was considered for analysis. The sum index was obtained by totaling the score of intensity and the percentage of positive cells. VEGF expression was defined as low (sum index ≤3) or high (sum index >3).

Statistical analysis. Statistical analysis was performed using the Statistical Analysis System, version 8.2 (SAS Institute Inc., Cary, NC, USA). Quantitative data were expressed in median values and interquartile range (IQR). The Kruskal Wallis and the Wilcoxon tests were used to test for differences in plasma VEGF-A and PSA levels between preoperative (clinical stage, pathological stage, Gleason score and surgical margins status), postoperative features (Gleason score, pathological stage and surgical margin status) and VEGF-A expression (low or high) in PCa tissue. The Spearman correlation coefficient was used to study the associations between quantitative variables. Associations between VEGF-A expression in PCa tissue and qualitative variables (clinical stage, pathological stage, Gleason score and surgical margins status) were determined using the Fisher's exact test. The Fisher's exact test was used to study the associations between preoperative Gleason score, PSA, MRI findings, postoperative Gleason score, pathological stage and surgical margin status.

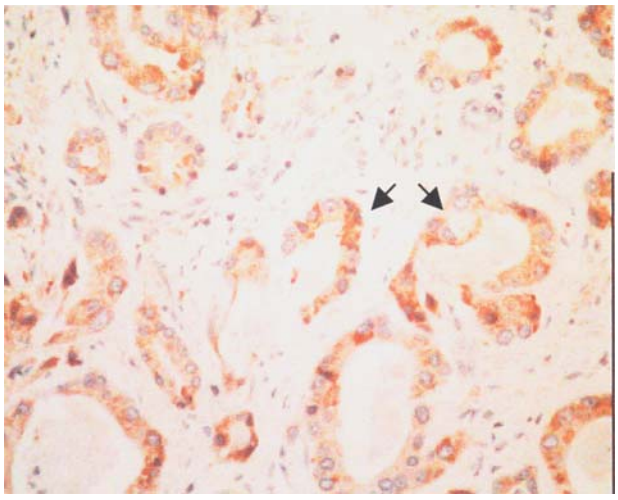

Figure 1. Case of PCa with high VEGF-A expression (intensity score 3) x400.

Results

The median plasma level of VEGF-A was 145.5 pg/ml (IQR 55.5-230; range 0-4,100). VEGF-A expression in PCa tissue could be determined in 89 cases. VEGF-A expression in PCa tissue was low in 53 patients, and high in the remaining 36. In patients with a positive staining, VEGF-A was detectable in stromal and in epithelial cells (Fig. 1).

There was no association between plasma VEGF-A values and patient age (p=0.22), prostate weight (p=0.46), total PSA (p=0.48), free/total PSA (p=0.9) or complexed PSA (p=0.51). Moreover, there was no association between VEGF-A expression in PCa tissue and patient age, prostate weight, different forms of PSA, or plasma VEGF-A (Table I).

Table II shows the plasma levels of VEGF-A according to preoperative and postoperative prognostic factors of PCa. There was no association between the plasma levels of VEGF-A and preoperative tumor characteristics. The plasma levels of VEGF-A were lower in patients with a high Gleason score on RP specimen (p=0.04). However, there was no association between the plasma levels of VEGF-A and pathological stage of PCa (p=0.57) or surgical margin status (p=0.48).

Table III shows the expression of VEGF-A in PCa tissue according to preoperative and postoperative tumor characteristics. There was no association between the expression of VEGF-A in PCa tissue and preoperative tumor characteristics. The expression of VEGF-A in PCa tissue was significantly higher in patients with a high Gleason score on RP specimen (p=0.01).

Table I. Association between VEGF-A expression in cancer tissue and quantitative parameters.

	Low VEGF-A expression	High VEGF-A expression	P-value[b]
Age median/ IQR[a] (years)	62/57-66	63/59-68	0.42
Prostate weight median/IQR[a] (gr)	44/38-54	46/33.5-56.5	0.42
Total PSA median/IQR[a] (ng/ml)	5.5/3.7-7.6	5.6/4-10.9	0.28
Free/total PSA median/IQR[a] (%)	14/11-19	11.5/8.5-19	0.19
Complexed PSA median/IQR[a] (ng/ml)	4.8/3.2-7	4.8/3.7-10.3	0.29
Plasma VEGF median/IQR[a] (pg/ml)	151/57-273	139/61-218	0.70

[a]Interquartile range. [b]Wilcoxon test.

Table II. Plasma levels of VEGF-A according to preoperative and postoperative tumor characteristics.

Tumor characteristics	N (%)	VEGF-A median/ IQR[a] (pg/ml)
Gleason score on biopsy		
≤6	59 (59)	182/75-232
3+4	24 (24)	80.5/5.5-218
4+3	11 (11)	71/28-110
>7	6 (6)	64/58-208
P-value[b]		0.13
Clinical stage of PCa		
T1c	71 (71)	163/36-250
T2a	23 (23)	89/57-159
T2b	6 (6)	460/0-511
P-value[b]		0.11
Percentage of positive biopsy cores		
<25	34 (34)	152/34-233
25-49	34 (34)	137.5/36-215
50-74	25 (25)	134/63-250
≥75	7 (7)	182/0-192
P-value[b]		0.96
Endorectal MRI findings		
No extracapsular extension	88 (88)	139/55.5-230.5
Suspected extracapsular extension	7 (7)	163/58-437
Extracapsular extension	5 (5)	154/0-208
P-value[b]		0.75
Gleason score on RP specimen		
≤6	52 (52)	189/89-233
3+4	24 (24)	139/45.5-373
4+3	16 (16)	62.5/19.5-123
>7	6 (6)	112.5/3-159
P-value[b]		0.04
Pathological stage of PCa		
pT0	2 (2)	122.5/36-209
pT2a	6 (6)	264.5/224-486
pT2b	22 (22)	139/58-328
pT2c	34 (34)	125/57-214
pT3a	34 (34)	152.5/54-215
pT3b	2 (2)	218.5/0-437
P-value[b]		0.57
Surgical margin status		
Positive	19 (19)	135/3-224
Negative	81 (81)	154/58-232
P-value[b]		0.48

[a]Interquartile range. [b]Kruskal-Wallis test.

Two factors were found to be associated with the pathological stage of PCa: the free/total PSA ratio and the post-operative Gleason score. The median free/total PSA ratio was 14% in patients with a pT2 tumor versus 11.5% in those with a pT3 tumor (p=0.04). The rates of extracapsular disease were 19.2, 50, 56.3 and 83.3% in patients with a Gleason score ≤6 or of 3+4, 4+3 and >7, respectively (p=0.002). Finally, the sole factor associated with surgical margin status was the pathological stage of PCa. Of the patients with positive surgical margins, 26.3% had a pT2 tumor and 73.7% had a pT3 tumor (p=0.001).

Discussion

The clinical impact of VEGF in metastatic PCa has been documented by several studies. Duque *et al* compared the plasma levels of VEGF-A in 54 patients with localized PCa, 26 patients with metastatic PCa, and 26 controls (4). They found a significant increase in VEGF-A in patients with metastases compared with patients with localized disease and controls. The role of VEGF in metastatic PCa was also suggested by George *et al*, who found in a series of 197 patients with hormone-refractory PCa that the plasma levels of VEGF-A were independently associated with overall survival (7). The evidence that VEGF plays a significant role in advanced PCa has led to the development of anti-angiogenic therapies. These targeted therapies include bevacizumab, a humanized VEGF-specific monoclonal antibody (8). Anti-angiogenic agents are still under investigation in clinical trials, but *in vivo* studies have reached promising conclusions (9).

Table III. Expression of VEGF-A in PCa tissue according to preoperative and postoperative tumor characteristics.

Tumor characteristics	Low VEGF-A expression N (%)	High VEGF-A expression N (%)
Gleason score on biopsy		
≤6	36 (67.9)	16 (44.4)
3+4	10 (18.9)	11 (30.5)
4+3	4 (7.5)	6 (16.7)
>7	3 (5.7)	3 (8.4)
P-value[a]		0.87
Clinical stage of PCa		
T1c	40 (75.4)	23 (63.9)
T2a	10 (18.9)	10 (27.8)
T2b	3 (5.7)	3 (8.3)
P-value[a]		0.47
Percentage of positive biopsy cores		
<25	17 (32.2)	10 (27.8)
25-49	19 (35.8)	11 (30.6)
50-74	13 (24.5)	12 (33.3)
≥75	4 (7.5)	3 (8.3)
P-value[a]		0.84
Endorectal MRI findings		
No extracapsular extension	48 (90.6)	29 (80.6)
Suspected extracapsular extension	5 (9.4)	7 (19.4)
P-value[a]		0.21
Gleason score on RP specimen		
≤6	34 (64.2)	12 (33.3)
3+4	12 (22.6)	12 (33.3)
4+3	7 (13.2)	7 (19.5)
>7		5 (13.9)
P-value[a]		0.01
Pathological stage of PCa		
pT2a	3 (5.7)	1 (2.8)
pT2b	12 (22.6)	7 (19.4)
pT2c	22 (41.5)	12 (33.3)
pT3a	15 (28.3)	15 (41.7)
pT3b	1 (1.9)	1 (2.8)
P-value[a]		0.38
Surgical margin status		
Positive	10 (18.9)	7 (19.4)
Negative	43 (81.1)	29 (80.6)
P-value[a]		1

[a]Fisher's exact test.

Although VEGF plays an important role in metastatic PCa, its impact in localized disease remains to be clarified. Our team has previously analysed the diagnostic value of VEGF for the early detection of PCa (10). We measured VEGF-A in 47 patients who underwent prostate biopsies on clinical and/or biological suspicion of PCa, and found that VEGF was not predictive of cancer on biopsies. This study relied on serum samples. Because VEGF measured in serum may be released from platelets on activation after venipuncture (11), we decided, in the current study, to measure VEGF in plasma.

Shariat *et al* analysed the plama levels of VEGF-A in 215 patients who underwent RP for clinically-localized PCa, in 9 patients with untreated metastatic PCa, and in 40 controls (5). In this study, preoperative levels of VEGF-A were significantly elevated in patients with Gleason score ≥7, in patients with extraprostatic stage, and in those with lymph node involvement. Moreover, patients with plasma levels of VEGF-A above the median had an increased risk of biochemical progression after RP. The authors concluded that preoperative levels of VEGF-A could help to predict the tumoral stage and the clinical outcome of patients. The results of our current study gave rise to different conclusions. Such discrepancy may be due to differing methods of VEGF measurement: we used a polyclonal antibody that is designed to measure all the isoforms of VEGF-A, whereas Shariat *et al* used an immunoassay technique that measures only two isoforms of VEGF-A (VEGF 121 and VEGF 165) (5).

A surprising finding in our study is that VEGF-A levels were lower in patients with a high Gleason score. However, this result should be considered with caution, owing to the small sample of patients with a high Gleason score. In fact, the vast majority of patients had a Gleason score of 6, and only 6 patients had a Gleason score >7; it is therefore difficult to analyse this parameter.

George *et al* measured plasma levels of VEGF-A before and after RP in 86 patients with clinically localized PCa (12). The median reduction in VEGF from before RP to after RP was only 20%. These results suggested that the changes in the post-RP VEGF values could be consistent with a non-cancerous source. As was the case in our study, there was no association between plasma levels of VEGF and pre-RP PSA, Gleason score on biopsy, or clinical stage of PCa.

We found that VEGF-A expression was strongly associated with the Gleason score of PCa. This marker could therefore play a prognostic role in localized PCa. To our knowledge, our current study is the first to analyse the prognostic value of VEGF-A expression in PCa. Some previous studies analysed the prognostic value of VEGF-C expression. VEGF-C causes proliferation of lymphatic endothelial cells and plays a role in tumor lymphangiogenesis (1). Li *et al* compared the expression of VEGF-C receptor (VEGFR-3) in benign prostate hyperplasia and in PCa (13). These authors found that VEGFR-3 was up-regulated in PCa. Moreover, they found a correlation between VEGFR-3 expression and preoperative PSA, Gleason score and lymph node metastases. Jennbacken *et al* found a higher expression of VEGF-C in patients with lymph node metastases than in those with localized PCa (14). Since our current report is the first to analyse the prognostic value of VEGF-A expression in PCa, our results need to be confirmed by further studies.

In our study the clinical outcome of patients was not analysed. Over the last decade, the oncological results of RP

have improved markedly, owing to better patient selection and enhanced technique (15). Furthermore, the interval between treatment and disease recurrence is often longer than two years. To determine whether VEGF-A has an effect on the risk of PCa recurrence and progression, a large cohort of patients with long-term follow-up is mandatory. A major prospective study that will analyse plasma levels of VEGF-A, VEGF-A expression in prostatic tissue, and patient outcome is currently under way in our department.

In summary, we found that plasma levels of VEGF-A were not associated with the established prognostic factors of localized PCa. On the contrary, the expression of VEGF-A in PCa tissue was strongly associated with the Gleason score of cancer. These findings suggest that the expression of VEGF-A in prostate tissue could have a prognostic impact in localized PCa. Because only sparse data are available in the literature, further evaluation of clinical outcome is mandatory to determine the prognostic value of VEGF expression in localized PCa.

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