The prognostic value of vascular endothelial growth factor (VEGF)-A and its receptor in clinically localized prostate cancer: a prospective evaluation in 100 patients undergoing radical prostatectomy

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Objectives: *To study the prognostic value of vascular endothelial growth factor (VEGF)-A and its receptor VEGFR-1 in localized prostate cancer.*
Methods: *One hundred patients undergoing radical prostatectomy (RP) for clinically localized prostate cancer were prospectively included. Plasma levels of VEGF-A were measured preoperatively. After intervention, tissue microarrays were built from the RP specimens. VEGF-A and VEGFR-1 expressions in prostate cancer tissue were determined using immunochemistry. Then the associations between plasma levels of VEGF-A, VEGF-A and VEGFR-1 expressions in prostate cancer tissue, and the outcome of patients were analyzed.*
Results: *After a median follow-up of 22 months, 14 patients experienced biological recurrence of prostate cancer. There was no correlation between plasma VEGF-A and the risk of recurrence following RP. Moreover, there was no correlation between VEGF-A expression or VEGFR-1 expression in prostate cancer tissue and the risk of recurrence after RP.*
Conclusions: *Plasma levels of VEGF-A, the expression of VEGF-A and that of VEGFR-1 in prostate cancer tissue did not affect patients outcome following RP. VEGF-A and its receptor VEGFR-1 may have no prognostic value in localized prostate cancer. Further studies with longer follow-up are mandatory to confirm these findings.*

Key Words: vascular endothelial growth factor, VEGF-A, radical prostatectomy

Introduction

Vascular endothelial growth factor (VEGF) is an important regulator of angiogenesis.

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Five forms of VEGF have been identified: VEGF-A, -B, -C, -D and -E.[1] VEGF-A plays a role in differentiation and proliferation of the endothelial cells of blood vessels. It is involved in the growth of many variants of tumors, including prostate cancer.[2] VEGF-A expression in prostatic tissue is associated with the presence of cancer, and it is higher in metastatic prostate cancer than in localized prostate cancer.[2] Moreover, some studies have shown a correlation between circulating levels of VEGF-A and the stage of prostate cancer. For example, Jones et

al noted, in a series of 78 men, that serum VEGF-A levels were significantly higher in patients with metastatic prostate cancer than in those with localized prostate cancer or controls.[3] These findings suggest that VEGF-A in prostatic tissue, as well as in blood, could have a prognostic impact in prostate cancer.

VEGFR-1 is one of the receptors for VEGF-A. The functions of VEGFR-1 have not been clearly determined. It participates in recruitment of endothelial cell progenitors, and activates growth factors from liver endothelial cells.[4] Preliminary in vivo studies have suggested that VEGFR-1 was a negative regulator of VEGF activity, but these studies were not confirmed by further investigations.[4] Although pathological studies show that VEGFR-1 is over-expressed in prostate cancer tissue,[5] clinical data regarding its prognostic impact are lacking.

Since only few studies have analyzed the clinical utility of VEGF-A and its receptor VEGFR-1 in patients with prostate cancer, we tried to determine their prognostic value in patients treated for localized prostate cancer. We measured the plasma levels of VEGF-A and we determined the tissue expressions of VEGF-A and VEGFR-1 in a cohort of patients undergoing radical prostatectomy (RP). Then we analyzed the associations between the plasma and tissue biomarkers and PSA recurrence.

Material and methods

Patient selection

One hundred consecutive patients (mean age 65.2 years; range 44 to 74) undergoing RP for clinically localized prostate cancer were prospectively enrolled in the study between June and November 2005. No patients had received hormone therapy or radiation therapy prior to surgical intervention. All patients had undergone pelvic CT scan, endorectal magnetic resonance imaging (MRI) and bone scintigraphy before RP. No patients had clinical or radiological evidence of lymph node or bone metastases. A standard bilateral pelvic lymphadenectomy was performed at the time of RP.

After RP, all patients had a PSA determination every 3 months during 1 year, then every 6 months in the absence of recurrence. Biological recurrence following RP was defined as postoperative PSA elevation ≥ 0.2 ng/ml, with a second confirmatory level of PSA of ≥ 0.2 ng/ml.

Assessment of plasma VEGF-A

Blood was taken from all patients the day before RP. VEGF-A was measured using an enzyme-linked immunosorbent assay (ELISA). Blood was spun at 2500 g for 10 minutes; then, the platelet-poor-plasma was removed and placed in a 5 ml tube, stored at –80°C and thawed just before testing. VEGF-A levels were determined in duplicate with 100 µl of each sample. For each patient, two measurements were therefore available. The mean value of both plasma VEGF-A levels was considered for analysis. According to their amino acid content, five 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 isoforms. Sex steroid hormones were not measured in this study.

Tissue microarrays

Slides from the 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 benign prostatic tissue.

Immunohistochemistry (VEGF-A and VEGFR-1)

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° in a 0.01 M citrate buffer (PH6) for 15 minutes. An anti-VEGF A-20 Mab (clone sc-152; Santa Cruz Biotechnology, California, USA) was used at the dilution of 1:200 during 1 hour. An anti-VEGFR-1 (anti-Flt-1, AF321) (R&D, Minneapolis, USA) was used at the dilution of 1:20 during 3 hours. After rinsing in PBS, the horse anti goat biotynylated, secondary antibody (BA9005) (Abcys, Paris, France) was applied for 30 minutes. A peroxydase complex was used (PK-6100). To visualize the reaction, sections were incubated with an AEC substrate chromogen (Dakocytomation, Copenhagen, Denmark) for 15 to 20 minutes 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

Two pathologists blinded for 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, Figure 1. Because

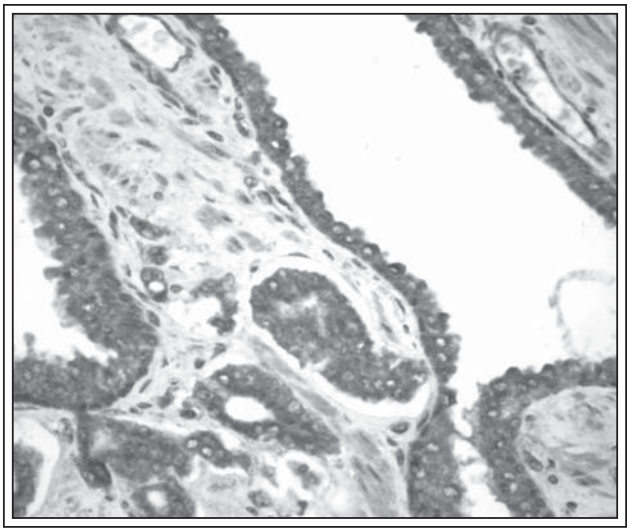

Figure 1a. Case of prostate cancer with moderate VEGF-A expression (intensity score 2) X 400.

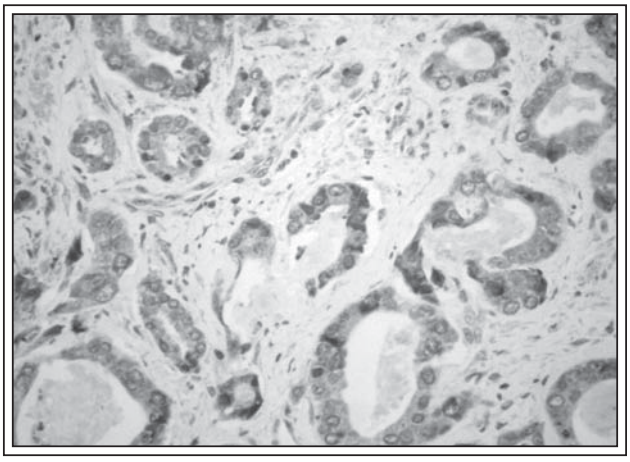

Figure 1b. Case of prostate cancer with high VEGFR-1 expression (intensity score 3) X 400.

of the duplicate nature of the arrays, two values were obtained for each patient. The highest intensity value was considered for analysis. When both pathologists found two different intensity scores for one patient, the highest score was considered for analysis.

Statistical analysis

Statistical analysis was performed using the Statistical Analysis System, version 8.2 (SAS Institute Inc., Cary, NC, USA). The Mann-Whitney test was used to compare plasma VEGF values, and VEGF-A and VEGFR-1 expressions between patients who experienced recurrence following RP and those who remained free of recurrence.

TABLE 1. **Characteristics of tumors**

	n (%)
Gleason score on RP specimen	
< 6	1 (1%)
6	51 (52%)
3 + 4	24 (24.5%)
4 + 3	16 (16.3%)
> 7	6 (6.2%)
Pathological stage	
pT0	2 (2%)
pT2	62 (62%)
pT3	36 (36%)
Surgical margins	
Positive	19 (19%)
Negative	81 (81%)

Results

Mean preoperative PSA was 7 ng/ml (range 1-32). Table 1 lists the characteristics of tumors on pathological examination. Two patients had a pT0 tumor. The rate of pT3 tumors was 36%, and that of positive surgical margins was 19%. None of the patients had lymph nodes involvement.

Plasma levels of VEGF-A could be measured in all patients. Median plasma level of VEGF-A was 145.5 pg/ml (IQR 55.5-230; range 0-4,100). For technical reasons, VEGF-A and VEGFR-1 expressions in prostate cancer tissue could not be determined in 11 patients. Table 2 shows the repartition of VEGF-A and VEGFR-1 expressions in the remaining 89 patients.

TABLE 2. **Repartition of VEGF-A and VEGFR-1 expressions in 89 RP specimens**

Intensity score	**n (%)**
VEGF-A	
0	9 (10.1%)
1	1 (1.1%)
2	4 (4.5%)
3	17 (19.1%)
4	22 (24.7%)
5	32 (40%)
6	4 (4.5%)
VEGFR-1	
1	10 (11.2%)
2	73 (82%)
3	6 (6.8%)

TABLE 3. **Correlation analysis between plasma VEGF, VEGF-A expression, VEGFR-1 expression and Gleason score on RP specimen**

	Gleason score < 7	Gleason score ≥ 7	Mann-Whitney (z)	p-value
Median plasma VEGF	120 [0-4005]	80 [0-4100]	0.1	0.09
Median VEGF-A expression	4 [0-6]	5 [0-6]	0.01	0.005
Median VEGFR-1 expression	2 [1-3]	2 [1-3]	0.73	0.36

Plasma levels of VEGF-A, as well as VEGF-A and VEGFR-1 expressions in prostate cancer tissue, were not correlated with preoperative PSA (Pearson's correlation, $p > 0.1$). However, VEGF-A expression in prostate cancer tissue was significantly higher in patients with high-grade disease (Gleason score ≥ 7), Table 3.

After a median follow-up of 22 months following RP, 14 patients experienced biological recurrence of prostate cancer. Of them, 10 received radiation therapy, and the remaining 4 patients were managed with active monitoring. No patients had evidence of disease progression at the time of analysis. Plasma levels of VEGF-A, expression of VEGF-A and expression of VEGFR-1 in prostatic tissue were not significantly different in patients who recurred and in those who remained free of recurrence, Table 4.

Discussion

To date, the clinical impact of VEGF-A in localized prostate cancer has not been clarified. VEGF-A may be measured either in blood or in prostatic tissue. In the current study, we found that plasma levels of VEGF-A had no prognostic value in patients undergoing RP. In a previous study, Shariat et al analyzed the plasma levels of VEGF-A in 215 patients who underwent RP, in 9 patients with untreated metastatic prostate cancer, and in 40 controls.[6] Among the patients treated with RP, preoperative levels of VEGF-A were significantly higher in those with Gleason score ≥ 7, in those with pT3 stage, and in those with lymph nodes involvement. Moreover, there was a correlation between plasma VEGF-A and the risk of biochemical progression following RP. Our current study was not consistent with these findings. This discrepancy could be attributed to differing methods of VEGF measurement. Indeed, we used an antibody that is designed to measure all isoforms of VEGF-A, whereas Shariat et al used an immunoassay technique that measures only two isoforms (VEGF 121 and VEGF 165). Another explanation may be our strict inclusion criteria. Indeed, we included a vast majority of low grade tumors, while in the series of Shariat et al a significant proportion of patients had more aggressive tumors.

Another finding of our study is that the expression of VEGF-A in prostate cancer tissue had no prognostic value in localized prostate cancer. Although we found an association between VEGF-A expression in prostate cancer tissue and the Gleason score of tumors, there was no correlation with the risk of biological recurrence following intervention. Several studies have analyzed

TABLE 4. **Correlation analysis between plasma VEGF, VEGF-A expression, VEGFR-1 expression and the risk of recurrence**

	Gleason score < 7	Gleason score ≥ 7	Mann-Whitney (z)	p-value
Median plasma VEGF	120 [0-4005]	80 [0-4100]	0.1	0.09
Median VEGF-A expression	4 [0-6]	5 [0-6]	0.01	0.005
Median VEGFR-1 expression	2 [1-3]	2 [1-3]	0.73	0.36

the clinical impact of VEGF-A expression in prostate cancer. Strohmeyer et al measured the expression of VEGF-A in 55 prostate cancers, and found a correlation between VEGF-A expression and tumor stage and grade.[7] In addition, they found that VEGF-A expression was associated with the risk of cancer progression during follow-up. West et al measured the expression of VEGF-A in 67 prostate cancers, and reported that VEGF immunoreactivity in the prostatic stroma was associated with reduced survival.[8] However, both these studies included tumors of all stages, most of them being locally-advanced or metastatic. Therefore, they could not determine the prognostic value of VEGF-A expression in localized disease. Our team has previously studied the prognostic value of VEGF-A expression in clinically localized prostate cancer.[9] In this study, we compared VEGF-A expression in the RP specimens of 17 patients who experienced cancer progression following RP and in 23 patients who remained free of recurrence. We found that VEGF-A expression was higher in patients who experienced progression. However, our study had two major limitations: it was retrospective, and it included a small number of patients. In the current study, patients were prospectively included and highly selected. All had a clinically localized prostate cancer, without evidence of extraprostatic extension on pelvic CT scan, endorectal MRI or bone scintigraphy. To our knowledge, our current study is the first to investigate the prognostic value of VEGF-A expression exclusively in clinically localized prostate cancer. Surprisingly, we found that VEGF-A expression was correlated with the Gleason score of tumors, but not with the patient's outcome. One major limitation of our study is its short follow-up. With a higher number of patients included and with a longer follow-up, the results of our study could have been different.

We also found that the expression of VEGFR-1 had no prognostic impact on patients outcome. A genetic study has suggested that the methylation of the VEGFR-1 gene is involved in prostatic carcinogenesis.[10] Moreover, a pathological study has shown an overexpression of VEGFR-1 in prostate cancer tissue.[11] These studies support the hypothesis that VEGFR-1 plays a role in prostate cancer development. However, the clinical impact of VEGFR-1 in patients treated for prostate cancer remains unknown. Further studies are therefore necessary to determine the prognostic utility of VEGFR-1 in prostate cancer.

In summary, the results of our current study suggest that plasma levels of VEGF-A, as well as VEGF-A and VEGFR-1 expressions in prostate cancer tissue, have no prognostic value in localized prostate cancer. However, VEGF-A and its receptors may have a prognostic value in more advanced disease. Recently, Green et al found, in a series of 50 patients receiving radiation therapy for locally-advanced prostate cancer (T3N0M0 stage), that VEGF expression on pretreatment diagnostic tumor biopsies was the only significant factor associated with disease-specific survival.[12] Similarly, the role of VEGF has been highly suggested in advanced prostate cancer. Duque et al compared the plasma levels of VEGF-A in 54 patients with localized prostate cancer, 26 patients with metastatic prostate cancer, and 26 healthy controls.[13] They found that patients with metastases had significantly higher plasma levels than those with localized tumors and controls. It is therefore likely that VEGF-A and its receptor VEGFR-1 have a prognostic impact in advanced prostate cancer, but not in localized disease.

Finally, the critical question remains whether VEGF can be used as a target to treat prostate cancer. Antiangiogenic therapies are currently under investigation in metastatic prostate cancer. In a preclinical model, Yazici et al showed that the blockade of the VEGFR-1 signaling pathways combined with chemotherapy could suppress the dissemination of metastases of human prostate cancer cells.[14] Several clinical trials investigating the benefit of anti-VEGF therapy in prostate cancer are ongoing in the United States and in Europe.

Conclusions

Plasma levels of VEGF-A, and the expression of VEGF-A and VEGFR-1 in prostate cancer tissue, as assayed in this study, did not predict tumor recurrence following RP for clinically localized prostate cancer. Additional studies with longer follow-up are necessary to validate these initial findings, as well as further studies on patients with locally-advanced and metastatic disease. □

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