Blackwell Publishing

Asian J Androl 2006; 8 (2): 169–175
DOI: 10.1111/j.1745-7262.2006.00120.x

AJA

·Original Article·

Increased expressions of vascular endothelial growth factor (VEGF), VEGF-C and VEGF receptor-3 in prostate cancer tissue are associated with tumor progression

Jie Yang[1], Hong-Fei Wu[1], Li-Xin Qian [1], Wei Zhang[1], Li-Xin Hua[1], Mei-Lin Yu[2], Zhen Wang[2], Zheng-Quan Xu[1], Yuan-Geng Sui[1], Xin-Ru Wang[3]

[1]Department of Urology, [2]Department of Pathology, First Affiliated Hospital of Nanjing Medical University, Nanjing 210029, China
[3]Key Laboratory, School of Public Health, Nanjing Medical University, Nanjing 210029, China

Abstract

Aim: To investigate the differences in microvessel densities (MVD) and the expressions of vascular endothelial growth factor (VEGF), VEGF-C and VEGF receptor-3 (VEGFR-3) between prostate cancer (PCa) tissues and adjacent benign tissues, and to explore the correlations among MVD, Jewett–Whitmore staging, Gleason scores and expressions of VEGF, VEGF-C and VEGFR-3 in the progression of PCa. **Methods:** An immunohistochemical approach was adopted to detect the expressions of CD34, VEGF, VEGF-C and VEGFR-3 in both cancer areas and peripheral benign areas of 71 primary prostatic adenocarcinoma specimens. A statistic analysis was then performed according to the experimental and clinic data. **Results:** Significantly upregulated expressions of VEGF, VEGF-C and VEGFR-3 were all found in malignant epithelium/cancer cells compared with adjacent benign epithelium ($P < 0.01$). Patients in stage D had a significantly higher score than patients in stage A, B or C when comparing the expression of VEGF-C or VEGFR-3 in the tumor area ($P < 0.01$). In addition, significant correlations were observed between Jewett–Whitmore staging and VEGF-C ($r_s = 0.738$, $P < 0.01$), clinical staging and VEGFR-3 ($r_s = 0.410$, $P < 0.01$), VEGF-C and Gleason scores ($r_s = 0.401$, $P < 0.01$), VEGFR-3 and Gleason scores ($r_s = 0.581$, $P < 0.001$) and MVD and VEGF ($r_s = 0.492$, $P < 0.001$). **Conclusion:** Increased expressions of VEGF and VEGF-C were closely associated with progression of PCa. The main contribution of increased VEGF expression for PCa progression was to upregulate MVD, which maintained the growth advantage of tumor tissue. However, the chief role of increased expressions of VEGF-C and VEGFR-3 was to enhance lymphangiogenesis and provide a main pathway for cancer cells to disseminate. *(Asian J Androl 2006 Mar; 8: 169–175)*

Keywords: prostatic neoplasms; vascular endothelial growth factor; vascular endothelial growth factor c; vascular endothelial growth factor receptor-3; angiogenesis; lymphangiogenesis

Correspondence to: Dr Hong-Fei Wu, M.D., Department of Urology, First Affiliated Hospital of Nanjing Medical University, Nanjing 210029, China
Tel: +86-25-8371-6603, Fax: +86-25-8372-4440
E-mail: wuhongfei6603@hotmail.com
Received 2005-09-10 Accepted 2005-11-29

1 Introduction

Progression of prostate cancer (PCa) is associated with the development of an adequate blood supply by means of angiogenesis and metastases to regional lymph

nodes or other structures and organs, such as bone, liver or lung, *etc*. Tumor cells release soluble angiogenic factors that induce neovascularization, without which nutrients and oxygen would not be available for tumors to grow more than 2–3 mm in diameter [1]. Genes encoding pro-angiogenic proteins are necessary for PCa progression from latency to clinical invasiveness. Vascular endothelial growth factor (VEGF), one of the pro-angiogenic factors, has been reported in association with angiogenesis in many kinds of tumors [1, 2]. Its upregulated expression induced by hypoxia is the crucial event leading to neovascularization [2] and may correlate with PCa growth and metastasis [3]. Furthermore, many clinical studies have indicated that metastasis by means of blood flow is more important than that using lymphatic circulation, in progression and prognosis of PCa [1]. In addition, Joseph and Isaacs [4] have reported that VEGF is regulated by androgen, suggesting a potential pathway by which androgen regulates prostate cancer growth.

One of the major pathways for PCa cells to disseminate is through the lymphatic system and patients with lymph node metastases have a poor prognosis [5]. At present, the underlying molecular mechanism of lymph node metastasis is poorly understood, even though lymph node metastasis is a major prognostic factor in many human tumors. Recently, VEGF-C has been implicated in the regulation of tumor lymphangiogenesis and enhancement of lymphatic invasion through the activation of VEGF receptor-3 (VEGFR-3) [6, 7]. Furthermore, VEGF-C also gains the possibility to interact with VEGFR-2, which may also stimulate angiogenesis [8].

Another important lymphangiogenic factor, VEGFR-3, is mainly restricted to lymphatic endothelial cells in adults, but its expression has also been found in endothelial cells of tumor blood vessels [7]. It has also been shown that metastasis of cancer cells through the lymphatic system could be blocked by a VEGF-3 receptor [9].

For earlier detection and more successful treatment of PCa, a better understanding of the molecular mechanisms involved in its progression is needed. So we examined differences in microvascular densities (MVD) and expressions of VEGF, VEGF-C and VEGFR-3 proteins in PCa tissues with different clinical stagings by an immunohistochemical approach and explored their possible associations with PCa progression.

2 Materials and methods

2.1 Tissue specimens

PCa samples from 71 primary adenocarcinoma cases were included in this study, after two pathologists had reviewed all specimens to exclude other pathological types. The samples were 10% formalin-fixed and paraffin-embedded. The median age of the patients was 72 years (range 55–82 years). All samples were obtained by transurethral resection of the prostate, radical prostatectomy or needle-biopsy at the First Affiliated Hospital of Nanjing Medical University (Nanjing, China) between 2002 and 2005. Iliacal lymph nodes were resected in order to judge whether there were metastases of lymph nodes in all cases treated with radical prostatectomy. History, rectal exam, computed tomography, magnetic resonance imaging and isotope scanning of the skeleton were combined to decide the clinical staging. Each section contained areas of adenocarcinoma with adjacent benign prostate tissue. The study was conducted with the approval of the ethical committee of Nanjing Medical University (Nanjing, China).

2.2 Immunohistochemistry

Immunostaining was carried out using a streptavidin–biotin–immunoperoxidase complex method [7] with 4 μm-thick sections, which had been deparaffinized, incubated with 3% H_2O_2 for 10 min to inactivate the endogenous peroxidase, and heated in citrate buffer solution (0.01 mmol/L, pH 6.0) for 15 min with a microwave oven to retrieve antigens. Normal goat serum was used as a blocking agent for 20 min at room temperature. Then the sections were incubated overnight at 4ºC with the following primary antibodies: mouse polyclonal anti-VEGF (1 : 100 dilution; Santa Cruz Biotechnology, Santa Cruz, CA, USA); mouse monoclonal anti-CD34, anti-VEGF-C and anti-VEGFR-3 (prediluted; Zhongshan Biotechnology, Beijing, China), respectively. After subsequent washes with phosphate-buffered saline (PBS; 0.01 mol/L, pH 7.3), the sections were treated with biotinylated secondary antibody for 20 min, washed and treated with streptavidin-enzyme conjugate. Diaminobenzidine was applied as the final chromogen, and nuclei were counterstained with Mayers hematoxylin solution to facilitate microscopic assessment. For the negative control, the primary antibody was replaced with PBS.

2.3 Evaluation of immunostaining

All slides were examined by two trained pathologists. Immunoreactivity for VEGF, VEGF-C and VEGFR-3 was

evaluated using a semiquantitative scoring system for intensity of stain and percentage of positive cells. Staining intensity was scored as follows: 0, none; 1, weak; 2, moderate; 3, strong. The positively stained area was expressed as the percentage of the whole field, and scored as: 0, none; 1, 0%–25%; 2, 25%–50%; 3, >50% positive cells. Then the immunoreactive scores for each specimen were calculated by multiplication of the values for the two parameters [10]. CD34-stained sections were used to calculate the capillary density. The specimens were observed microscopically, and areas with the densest microvasculature (hot spots) were selected under high-power magnification (× 200). The number of positively stained vessels in five fields was counted, and the mean value was defined as the MVD for each sample.

2.4 Statistical analysis

Epidata 3.0 software (Lauritsen JM & Bruus M. EpiData, Odense, Denmark) was used to input the data, and SPSS version 12.0 for Windows (SPSS, Nanjing, China) was used for statistical analysis. Comparisons between two groups were made with the Mann–Whitney *U*-test or Wilcoxon signed-ranks test, as appropriate. Associations among MVD, clinical staging and expressions of VEGF, VEGF-C and VEGFR-3 were analyzed using the Spearman rank correlation coefficient test. Correlation coefficient (r_s) value > 0.4 and $P < 0.05$ were considered significant in the Spearman rank correlation coefficient test, and all of the *P* values were based on two-sided testing.

3 Results

3.1 Expression of CD34

CD34, mainly expressed in blood vessel endothelial cells, was used to act as the marker of capillary (Figure 1A). The median MVD with different Jewett–Whitmore staging was: 68.0/high-power field in stage A; 65.5 in B; 86.0 in C; 117.5 in D. By a Mann–Whitney *U*-test, we found that the MVD in stage D was significantly higher than that in stage A ($P = 0.005$) or stage B ($P < 0.001$), but there was no obvious difference between stages C and D ($P = 0.052$). In addition, we discovered that there also existed significant differences between stages C and A ($P = 0.038$), and stages C and B ($P = 0.047$), but not between stages A and B ($P = 0.851$; Figure 1B).

3.2 Expression of VEGF

VEGF expression was mainly located in the cytoplasm and the plasma membrane of cancer cells and vessel endothelial cells (Figure 2A, D). The immunoreactivity of VEGF was heterogeneously distributed, and there was a broad variation in staining intensity. Diffuse immunoreactivity of variable intensity was also observed in

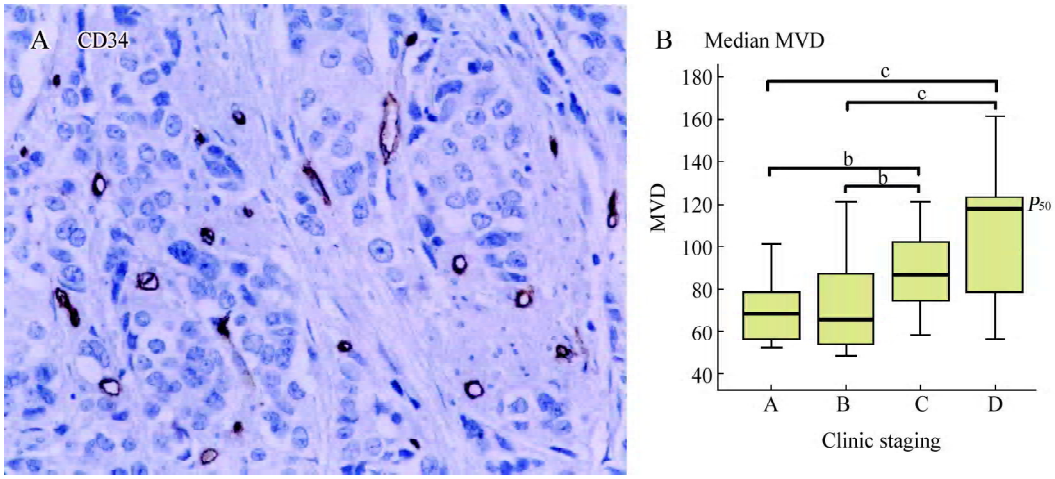

Figure 1. (A): Immunoperoxidase staining of CD34 in prostate cancer tissue specimens (brown denotes positive, × 400). (B): Box plot of microvessel densities (MVD) with different Jewett-Whitmore staging. $^bP < 0.05$; $^cP < 0.01$. Comparisons of MVD between two groups with different Jewett–Whitmore staging by a Mann–Whitney *U*-test. P_{50}, percentile 50 or median.

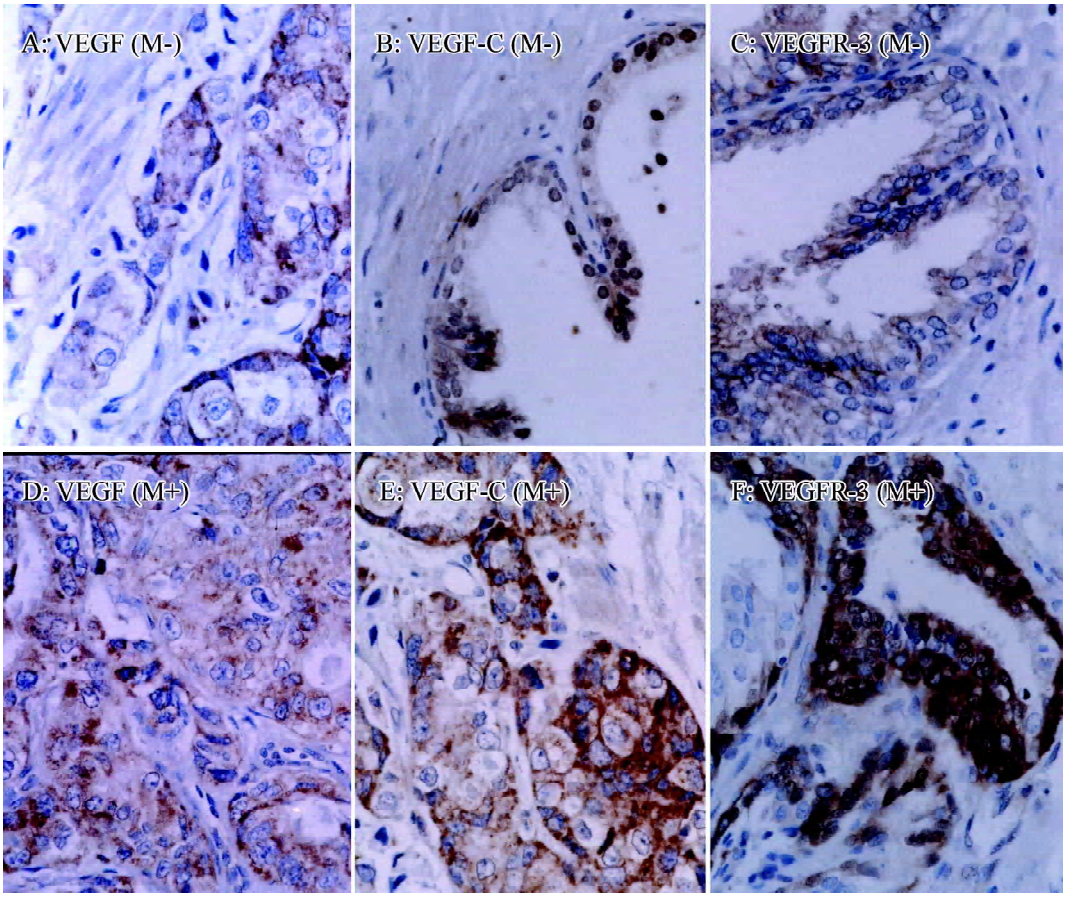

Figure 2. Immunoperoxidase staining of vascular endothelial growth factor (VEGF), VEGF-C and VEGF receptor-3 in prostate cancer specimens (brown denotes positive, × 400). M–, metastasis negative; M+, metastasis positive.

periglandular stroma. According to the immunostaining scores calculated, we discovered that the immunoreactivity for VEGF in adenocarcinoma parts of the samples was significantly more intense than that of the benign parts of the samples ($P = 0.001$, Table 1). But there was no obvious difference in immunoreactivity between patients with metastasis of lymph nodes or other organs (stage D) and patients without metastasis (stage A, B or C) ($P = 0.172$, Table 2).

3.3 Expression of VEGF-C

The expression of VEGF-C in glandular epithelium/cancer cells was also located in the cytoplasm (Figure 2B, E). Except for being expressed by glandular epithelium/tumor cells, VEGF-C was also expressed by some vascular endothelial cells, smooth muscle cells of arter-

Table 1. Comparisons of immunostaining scores between the carcinoma area and the benign area in each prostate cancer sample by a Wilcoxon signed-ranks test. $^cP < 0.01$. N, Number of samples; T, Sum of ranks; VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor.

	Immunostaining scores									
	0	1	2	3	4	6	9	N	T	*P*
VEGF										
Carcinoma area	11	8	36	7	5	4	0	71		
Benign area	24	16	19	12	0	0	0	71	494.0	0.001^c
VEGF-C										
Carcinoma area	7	12	19	9	10	14	0	71		
Benign area	21	20	17	7	6	0	0	71	74.5	0.000^c
VEGFR-3										
Carcinoma area	10	6	20	8	20	7	0	71		
Benign area	30	6	22	9	3	1	0	71	144.0	0.000^c

Table 2. Comparisons of immunostaining scores in malignant epithelium or cancer cells between Jewett–Whitmore stages A, B or C and stage D by a Mann–Whitney *U*-test. [c]*P* < 0.01. N, Number of samples; T, Sum of ranks; VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor.

	Immunostaining scores									
	0	1	2	3	4	6	9	N	T	*P*
VEGF										
Stage A, B or C	10	5	28	5	4	1	0	53		
Stage D	1	3	8	2	1	3	0	18	744.0	0.172
VEGF-C										
Stage A, B or C	7	11	18	7	7	3	0	53		
Stage D	0	1	1	2	3	11	0	18	995.5	0.000[c]
VEGFR-3										
Stage A, B or C	10	5	17	4	16	1	0	53		
Stage D	0	1	3	4	4	6	0	18	244.0	0.002[c]

ies and macrophages. The majority of tumor cells expressed VEGF-C, but with different intensities. In contrast, benign epithelium in peripheral areas of tumor tissue showed a weaker staining pattern than malignant epithelium ($P < 0.001$, Table 1). Even if the majority of tumor areas of specimens were positive for VEGF-C, the staining intensity differed markedly among the patients. Using the Mann–Whitney *U*-test, we found that patients in stage D had a significantly higher score than patients in stages A, B or C when comparing VEGF-C expression in the cancer area ($P < 0.001$, Table 2).

3.4 Expression of VEGFR-3

VEGFR-3 was expressed by lymphatic endothelial cells and partial blood vessel endothelial cells and, interestingly, also by the malignant epithelial cells (Figure 2C, F). Most of the normal prostate epithelial cells were negative for VEGFR-3, so the immunoreactivity for VEGFR-3 in malignant epithelial parts of samples was significantly more intense ($P < 0.001$, Table 1). When comparing cancer areas of samples in stage A, B or C, we found that expression of the receptor was higher in patients with stage D ($P = 0.002$, Table 2).

3.5 Correlations

We analyzed the correlations among MVD, Gleason scores, Jewett–Whitmore staging and expressions of VEGF, VEGF-C and VEGFR-3 in tumor areas of all specimens by a Spearman rank correlation coefficient test. Significant correlations were observed between Jewett–Whitmore staging and MVD ($r_s = 0.481$, $P < 0.001$), clinical staging and VEGF-C ($r_s = 0.738$, $P < 0.001$), clinical staging and VEGFR-3 ($r_s = 0.410$, $P < 0.001$), clinical staging and Gleason scores ($r_s = 0.471$, $P < 0.001$), Gleason scores and MVD ($r_s = 0.733$, $P < 0.001$), Gleason scores and VEGF-C ($r_s = 0.401$, $P = 0.001$), Gleason scores and VEGFR-3 ($r_s = 0.581$, $P < 0.001$) and MVD and VEGF ($r_s = 0.492$, $P < 0.001$). No other significant result was obtained (Table 3).

4 Discussion

The progression of tumors includes invasive growth to periphery tissue and metastasis of lymph nodes through the lymphatics or metastasis of distant organs by means of blood flow. Lymphatic invasion is the major and early manner for PCa cells to disseminate. Pelvic lymph node involvement is the first sign of metastasis in many PCa cases, then the metastases of other structures and organs, including bone, liver and lung, by means of blood circulation [11]. In this process, tumor cells may produce

Table 3. Correlations among microvessel densities (MVD), Gleason scores, Jewett–Whitmore staging and expressions of vascular endothelial growth factor (VEGF), VEGF-C and VEGF receptor-3 (VEGFR-3) in tumor areas of prostate cancer specimens. Correlation coefficient (r_s) > 0.4 and $P < 0.05$ are considered significant (values in bold).

	Jewett–Whitmore staging					Gleason score			
	MVD	VEGF	VEGF-C	VEGFR-3	Gleason score	MVD	VEGF	VEGF-C	VEGFR-3
r_s	0.481	0.138	0.738	0.410	0.471	0.733	0.400	0.401	0.581
P	0.000	0.253	0.000	0.000	0.000	0.000	0.001	0.001	0.000
	MVD			VEGF		VEGF-C			
	VEGF	VEGF-C	VEGFR-3	VEGF-C	VEGFR-3	VEGFR-3			
r_s	0.492	0.395	0.350	0.087	0.162	0.181			
P	0.000	0.001	0.003	0.468	0.177	0.131			

many factors that promote angiogenesis and lymphangiogenesis in order to gain sufficient nutrients and oxygen as well as pathways for dissemination. Recent studies on the VEGF/VEGFR-2 axis and VEGF-C/VEGR-3 axis have begun to elucidate the molecular mechanisms involved in angiogenesis and lymphangiogenesis [6-9, 13-15]. Our work also suggests that the increased expression of VEGF, VEGF-C and VEGFR-3 proteins may play a crucial role in the progression of PCa.

At present, many studies have demonstrated that the expression of VEGF, VEGF-C and VEGFR-3 are all increased in human PCa tissue compared with normal prostate tissue or benign prostatic hyperplasia tissue [3, 12, 13]. Our work also found the significantly upregulated expression of the three proteins in malignant epithelium/cancer cells when referenced to adjacent benign epithelium. Peripheral benign glands of cancer foci were usually weakly stained for VEGF, VEGF-C or VEGFR-3. This might indicate that the three proteins are all upregulated in the transition process from benign glands to cancer foci.

In addition, the immunostaining for VEGF was confined almost exclusively to the basal cell layer in peripheral benign epithelium, but was seen in all neoplastic secretory cells as well as the basal cell layer in malignant glands, which was also discovered in Kollermann and Helpap's study [13]. By an analysis of Spearman rank correlation coefficient tests, increased VEGF expression was found to be positively correlated with MVD as well as cell differentiation (Gleason score), though the correlation coefficient was only 0.400. These results strongly suggested that the PCa growth advantage was a consequence of angiogenic stimulation conferred by upregulated expression for VEGF in malignant epithelium, at least in part, and tumor cells with worse differentiation had higher expression of VEGF, which finally resulted in higher MVD and more marked growth advantage. Otherwise, we did not find a significant correlation between VEGF expression and Jewett–Whitmore staging ($r_s = 0.138$, $P = 0.253$), which might be due to the spreading of PCa cells by means of blood flow was not the primary and main manner.

A prerequisite for lymph node metastasis is the formation of lymphatic vessels in the tumor foci, so-called lymphangiogenesis. Studies on clinical specimens have shown that the expression of VEGF-C is upregulated in many types of human tumors and also related to the appearance of lymph node metastases [14, 15]. Several experimental animal studies also strongly suggested that lymphangiogenesis did occur in the presence of VEGF-C, and VEGF-C could not only induce hyperplasia of existing lymphatic vessels [16], but could also stimulate proliferation of new lymphatic vessels [17]. But in prostate cancer, the reports are somewhat contradictory. Tsurusaki *et al*. [6] found a significant relationship between expression of VEGF-C and lymph node metastasis, whereas Zeng *et al*. [7] did not. Our results were consistent with those of Tsurusaki *et al*. [6] and showed a significant difference in staining scores for VEGF-C between patients with metastasis (stage D) and patients without metastasis (stage A, B or C) by a Mann–Whitney *U*-test. Furthermore, by a Spearman rank correlation coefficient test we also discovered significantly positive correlations between expression for VEGF-C and Jewett–Whitmore staging or the Gleason score. But the correlation lying in between VEGF-C expression and MVD was not significant ($r_s = 0.395$, $P = 0.001$). However, Cao *et al*. reported that VEGF-C was also able to stimulate angiogenesis by interaction with VEGFR-2 [8], which might be due to the chief role of VEGF-C in enhancing lymphangiogenesis instead of angiogenesis. In addition, some experimental evidence has suggested that lymphatic endothelial cells could attract tumor cells by secreting chemokines, and therefore actively promote lymphatic metastasis [18, 19]. So we could suppose that VEGF-C might finally enhance metastasis by increasing the vascular permeability and stimulating the proliferation of lymphatic endothelial cells that increased the contact area between invading tumor cells and lymphatic endothelium.

Zeng *et al*. [7] reported a significant relationship between expression of VEGFR-3 and PCa metastasis, which our results are consistent with. We not only found that the immunoreactivity for VEGFR-3 in malignant epithelial parts of samples was significantly more intense, but also found that the expression of the receptor was higher in patients with stage D. Moreover, we also discovered significantly positive correlations between expression for VEGFR-3 and Jewett–Whitmore staging or Gleason scores. These results all proved the important role of VEGFR-3 in PCa progression and lymphangiogenesis.

But the statistical analysis did not show the significant correlation of expression between VEGF-C and VEGFR-3, even though VEGF-C mediates its effect through its receptor, VEGFR-3. This might imply that the increased expression of VEGF-C and VEGFR-3 was not parallel or co-instantaneous, a point that needs further research. Together with our findings, the expres-

sion of VEGFR-3 in malignant epithelial cells suggests a possible autocrine pathway of VEGF-C, which is mediated by the VEGF-C/VEGFR-3 axis and might play a fundamental role for metastasis of tumor.

In conclusion, the results of this study demonstrated that increased expression of VEGF, VEGF-C and VEGFR-3 were closely associated with progression of PCa. The main contribution of VEGF for PCa progression was to stimulate angiogenesis and upregulate MVD, which maintained the marked growth advantage of tumor tissue compared with adjacent benign tissue. However, the chief role of VEGF-C and VEGFR-3 in PCa progression was to enhance lymphangiogenesis and provide a main route for cancer cells to disseminate, although VEGF-C also had a weakly proangiogenic effect.

Acknowledgment

We thank Ms. Mei-Lin Yu and Mr. Zhen Wang (Department of Pathology, First Affiliated Hospital of Nanjing Medical University) for their technical assistance. This study was supported by the National Basic Research Program of China (No.2002CB512908) and the Natural Science Fund of Jiangsu Province, China (BK2005438).

References

1 Nicholson B, Theodorescu D. Angiogenesis and prostate cancer tumor growth. J Cell Biochem 2004; 91: 125–50.
2 Claffey KP, Brown LF, Del Aguila LF, Tognazzi K, Yeo KT, Manseau EJ, *et al.* Expression of vascular permeability factor/vascular endothelial growth factor by melanoma cells increases tumor growth angiogenesis and experimental metastasis. Cancer Res 1996; 56: 172–81.
3 Latil A, Bieche I, Pesche S. Valeri A, Fournier G, Cussenot O, *et al.* VEGF overexpression in clinically localized prostate tumors and neuropilin-1 overexpression in metastatic forms. Int J Cancer 2000; 89: 167–71.
4 Joseph IB, Isaacs JT. Potentiation of the antiangiogenic ability of linomide by androgen ablation involves down-regulation of vascular endothelial growth factor in human androgen-responsive prostatic cancers. Cancer Res 1997; 57: 1054–7.
5 Daneshmand S, Quek ML, Stein JP, Lieskovsky G, Cai J, Pinski J, *et al.* Prognosis of patients with lymph node positive prostate cancer following radical prostatectomy: longterm results. J Urol 2004; 172: 2252–5.
6 Tsurusaki T, Kanda S, Sakai H, Kanetake H, Saito Y, Alitalo K, *et al.* Vascular endothelial growth factor-C expression in human prostatic carcinoma and its relationship to lymph node metastasis. Br J Cancer 1999; 80: 309–13.
7 Zeng Y, Opeskin K, Baldwin ME, Horvath LG, Achen MG, Stacker SA, *et al.* Expression of vascular endothelial growth factor receptor-3 by lymphatic endothelial cells is associated with lymph node metastasis in prostate cancer. Clin Cancer Res 2004; 10: 5137–44.
8 Cao Y, Linden P, Farnebo J, Cao R, Eriksson A, Kumar V, *et al.* Vascular endothelial growth factor C induces angiogenesis *in vivo*. Proc Natl Acad Sci USA 1998; 95: 14389–94.
9 He Y, Kozaki K, Karpanen T, Koshikawa K, Yla-Herttuala S, Takahashi T, *et al.* Suppression of tumor lymphangiogenesis and lymph node metastasis by blocking vascular endothelial growth factor receptor 3 signaling. J Natl Cancer Inst 2002; 94: 819–25.
10 Ichinoe M, Mikami T, Shiraishi H, Okayasu I. High microvascular density is correlated with high VEGF, iNOS and COX-2 expression in penetrating growth-type early gastric carcinomas. Histopathology 2004; 45: 612–8.
11 Carrol PR, Lee KL, Fuks ZY, Kantoff PW. Cancer of the prostate. In: DeVita VT, Hellman S, Rosenberg SA, ed. Cancer: Principles and Practice of Oncology. Philadelphia: Lippincott Williams & Wilkins, 2001.p1419–25
12 Stefanou D, Batistatou A, Kamina S, Arkoumani E, Papachristou DJ, Agnantis NJ. Expression of vascular endothelial growth factor (VEGF) and association with microvessel density in benign prostatic hyperplasia and prostate cancer. In Vivo 2004; 18: 155–60.
13 Kollermann J, Helpap B. Expression of vascular endothelial growth factor (VEGF) and VEGF receptor Flk-1 in benign, premalignant, and malignant prostate tissue. Am J Clin Pathol 2001; 116: 115–21.
14 Yonemura Y, Fushida S, Bando E, Kinoshita K, Miwa K, Endo Y, *et al.* Lymphangiogenesis and the vascular endothelial growth factor receptor (VEGFR)-3 in gastric cancer. Eur J Cancer 2001; 37: 918–23.
15 Niki T, Iba S, Tokunou M, Yamada T, Matsuno Y, Hirohashi S. Expression of vascular endothelial growth factors A, B, C, and D and their relationships to lymph node status in lung adenocarcinoma. Clin Cancer Res 2000; 6: 2431–9.
16 Jeltsch M, Kaipainen A, Joukov V, Meng X, Lakso M, Rauvala H, *et al.* Hyperplasia of lymphatic vessels in VEGF-C transgenic mice. Science 1997; 276: 1423–5.
17 Oh SJ, Jeltsch MM, Birkenhager R, McCarthy JE, Weich HA, Christ B, *et al.* VEGF and VEGF-C: specific induction of angiogenesis and lymphangiogenesis in the differentiated avian chorioallantoic membrane. Dev Biol 1997; 188: 96–109.
18 Muller A, Homey B, Soto H, Ge N, Catron D, Buchanan ME, *et al.* Involvement of chemokine receptors in breast cancer metastasis. Nature 2001; 410: 50–6.
19 Kriehuber E, Breiteneder-Geleff S, Groeger M, Soleiman A, Schoppmann SF, Stingl G, *et al.* Isolation and characterization of dermal lymphatic and blood endothelial cells reveal stable and functionally specialized cell lineages. J Exp Med 2001; 194: 797–808.