The location of lymphangiogenesis is an independent prognostic factor in rectal cancers with or without preoperative radiotherapy

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Abstract

Background: Lymphangiogenesis and angiogenesis are essential for tumour development and progression. The lymphatic vessel density (LVD) and blood vessel density (BVD) and their relationship to outcome have been studied extensively, however the clinical significance of the location of LVD/BVD in tumour is not known. In the present study, the location and degree of LVD/BVD and their relationship to preoperative radiotherapy (RT), clinicopathological, histopathological and biological factors were studied in rectal cancer patients participating in a Swedish clinical trial of preoperative RT.

Patients and methods: The location and degree of LVD/BVD were analysed in primary tumours ($n = 138/140$) and in their subgroups of non-RT ($n = 74$) and RT ($n = 64/66$). Further, the degree of LVD/BVD was examined in the corresponding distant normal mucosa ($n = 35/31$) and adjacent normal mucosa ($n = 72/91$). All sections were immunohistochemically examined by using D2-40 and CD34 antibodies.

Results: In the whole series of the patients, a higher LVD at the periphery was related to negative p53 expression ($P = 0.03$) and favourable survival independent of tumour–node–metastasis stage, differentiation and p53 expression ($P = 0.03$). LVD was increased in p53-negative tumours after RT ($P = 0.01$).

Conclusion: LVD at the periphery of the tumour was an independent prognostic factor in rectal cancer patients.

Introduction

The onset of new blood vessel growth in tumours can be sudden, this reaction is initiated by hypoxia in tumour cells that leads to increased production of vascular endothelial growth factor (VEGF)-A, which stimulates the proliferation, migration and maturation of endothelial cells into capillary tubes. Whereas the development of blood vessels has been studied extensively, relatively little is known about the development of lymphatic vessels. Recently, it was shown that VEGF-C and -D regulate lymphatic vessel growth in bladder and gastric tumours [1, 2]. Earlier studies have shown a positive relationship between a high lymphatic

vessel density (LVD) or microvessel density (MVD) and poor prognosis in many types of cancers [1, 3, 4]. It was also shown that the LVD and MVD increased from normal mucosa to tumour [5, 6] and that the MVD decreased from tumour periphery to the intermediate and inner tumour area [3]. A higher LVD peritumoural (PT) compared with intratumoural (IT) has been found in breast and colorectal cancer [7, 8]. Few studies have investigated the location of the LVD in tumours and their relationship to clinical outcome. Bono et al. [7] found that PT was related to better survival in breast cancer while others found the reverse relationship [9]. As our knowledge, the location of MVD in relation to prognosis has not been studied.

The importance of angiogenesis and lymphangiogenesis and their response to radiotherapy (RT) has been recently raised. Coen et al. [10] showed that RT destroyed the integrity of the vascular structure and decreased the MVD, while LVD was found to increase after RT [11]. Since the lymphatic and blood system cooperate to facilitate the environment around tumour cells and play a central role in the metastatic spread of cancer, it is important to evaluate the responses of both the lymphatic and capillary network to RT. The aim of our present study was to investigate if degree and location of LVD/blood vessel density (BVD) were related to RT, clinicopathological (sex, age, tumour–node–metastasis (TNM) stage, differentiation grade, local recurrence and distant recurrence), histopathological (inflammatory infiltration and fibrosis) and biological factors (apoptosis and p53) in rectal cancer with or without RT.

Patients and Methods

This study included patients from the south-east Swedish Health Care region who participated in a Swedish clinical trial of preoperative RT from 1987 to 1990 [12]. All patients had rectal adenocarcinoma. The patients included in the study of LVD were 138 primary rectal cancers of which 74 underwent tumour resection alone and 64 preoperative RT before surgery, 35 with distant normal mucosa and 72 with adjacent normal mucosa. The patients included study of BVD were 140 primary rectal cancers of which 74 underwent tumour resection alone and 66 preoperative RT before surgery, 31 with distant normal mucosa and 91 with adjacent normal mucosa.

The required informed consent was given by all participants. RT was given with 25 Gy in five fractions during a median of 6 days (range 5–12 days). Surgery was then carried out for a median of 3 days (range 1–13 days) after RT. None of the patients received adjuvant chemotherapy before or after surgery. The mean age of the patients was 67 years (range 36–85 years) and the median follow-up was 86 months (range 0–193 months). Other characteristics of the patients and tumours are present in Table 1.

The histopathological factors (inflammatory infiltration and fibrosis) were analysed on sections stained with hematoxylin–eosin [13], the biological factors as apoptotic cells were detected by the TUNEL assay [14] and expressions of p53 [15] were determined by immunohistochemistry. The data were taken from our previous studies carried out at our laboratory.

Table 1: Number of patients with or without RT for LVD/BVD and their characteristics

Characteristics	LVD/BVD, *n* = 138/140	
	Non-RT (%)	RT (%)
Gender		
Male	43/43 (58/58)	40/41 (62/62)
Female	31/31 (42/42)	24/25 (38/38)
Age (years)		
≤67	31/30 (42/40)	28/29 (44/44)
>67	43/44 (58/60)	36/37 (56/56)
pTNM		
I	19/20 (26/27)	21/22 (33/33)
IIA	19/19 (26/26)	20/20 (31/30)
IIIA	8/8 (11/11)	1/1 (2/2)
IIIB	11/11 (15/15)	11/12 (17/18)
IIIC	12/11 (16/15)	4/4 (6/6)
IV	5/5 (6/6)	7/7 (11/11)
Differentiation		
Good	5/5 (7/7)	5/5 (8/8)
Moderate	53/53 (72/72)	38/40 (59/60)
Poor	16/16 (21/21)	21/21 (33/32)
Surgical type		
Rectal amputation	37/37 (50/50)	25/27 (39/41)
Anterior resection	37/37 (50/50)	39/39 (61/59)
Resection margin		
Tumour free	72/72 (97/97)	59/61 (92/92)
Tumour	2/2 (3/3)	5 (8/8)
To anal verge (cm)		
Mean	8.5	7.6

RT, radiotherapy; LVD, lymphatic vessel density; BVD, blood vessel density; pTNM, pathological tumour–node–metastasis.

immunohistochemistry

Five-micrometre paraffin-embedded sections were deparaffinised in xylene, rehydrated with a graded series of ethanol to water. The sections were cooked with Tris–EDTA buffer (pH 9.0) for D2-40 in a high-pressure cooker for 10 min and for CD 34 in a calibration bath (Julabo TW8, Seelbach, Germany) at 99°C for 40 min. Following preincubation in methanol with 0.3% H_2O_2 for 20 min, the sections were incubated with protein block (Dako, Carpinteria, CA) for 10 min and then incubated with mouse monoclonal D2-40 antibody (prediluted; Abcam, Cambridge, UK) or mouse monoclonal anti-CD34 antibody (18 μg/ml; Dako) at room temperature for 30 min. After washing in phosphate-buffered saline (pH 7.4),

the sections were incubated with anti-mouse secondary antibody (Dako) at room temperature for 25 min. Subsequently, the sections were subjected to 3,3′-diaminobenzidine tetrahydrochloride for 8 min and then counterstained by haematoxylin 5-μm sections from paraffin-embedded surgical specimen. Positive and negative controls were included in each staining run. In all staining procedures, the positive controls showed clear staining but no staining in the negative controls.

assesed MVD

MVD was assessed by counting microvessel in primary tumours and normal mucosa immunostained for D2-40 or CD34 antigen under light microscope on the basis of proposed standard method for MVD assessment given by the first international consensus [16] and expressed as the number of vessels per ×200 field, corresponding to an optical field of 0.74 mm^2. Briefly, the D2-40- or CD34-stained sections were initially scanned at low power (×40 and ×100) and the areas of specimens having the highest number of capillaries and small venules stained by D2-40 or CD34 (hot spots) were selected. Subsequently, microvessel counting was carried out in three fields of the hot spots, at ×200 magnification (×20 objective and ×10 ocular) and the mean value of the three fields at ×200 for each case was used for further analysis. Any brown-stained endothelial cells or cell cluster clearly separated from adjacent microvessels, tumour cells and other connective tissue elements were considered as a single countable vessel. Neither vessel lumina nor the presence of red blood cells were needed to define a microvessel. Large vessels with a muscular layer (artery) and microvessels in the areas with necrosis (because vascularisation in these areas could be related to the inflammatory reaction and not to the presence of a tumour), ulceration or intense inflammation within tumours were excluded and no cut-off calibre size was used for small microvessels and venules.

In previous studies on breast and head and neck cancer, the location of LVD was analysed IT and PT [7, 9]. Since the histopathological structure for colorectal tumours differs (has a luminal border) from breast and head and neck tumours and one study on colorectal cancer showed that tumour cells at the periphery of the whole tumour were related to a better outcome [17], we wanted to go further and examine LVD/BVD at the tumour periphery separately. By following a study of Kuokourakis et al. [3], the location of the hot spots in primary rectal tumours was examined either in the periphery, the inner tumour area or invasive margin. Two or three hot spots with the same location were combined and used for further analysis. The number of patients and their LVD/BVD location in tumours are presented in Table 2.

The microvessel count was carried out by two of the authors (AH and JG), using a double-headed light microscope simultaneously. Both authors agreed on the identification of each countable microvessel without any knowledge of the clinical outcomes and clinicopathological features. In the case of discrepancy (13%), a recount was taken to reach an agreement.

statistical analysis

An independent and a dependent student's *t*-tests were used to estimate the differences between LVD/BVD expression in normal mucosa and tumour. The chi-square method was used to test for the vessel location and an independent student's *t*-test for the degree of LVD/BVD in tumours. Cox proportional hazard model was used to estimate relationship

between the LVD/BVD expression and patient's survival in univariate and multivariate analyses. Survival curves were computed according to Kaplan–Meier method. Test were two-sided, and P <0.05 was considered statistically significant.

Table 2: Number of patients and their LVD/BVD location in tumours with or without RT

Characteristics	Non-RT (%)	RT (%)
LVD		
Periphery	14 (19)	11 (17)
Inner tumour area	40 (54)	32 (50)
Invasive margin	14 (19)	16 (25)
Negative cases	6 (8)	5 (8)
BVD		
Periphery	45 (61)	43 (65)
Inner tumour area	25 (34)	22 (33)
Invasive margin	4 (5)	1 (2)
Negative cases	0 (0)	0 (0)

LVD, lymphatic vessel density; BVD, blood vessel density; RT, radiotherapy.

Results

location of D2-40 and CD34 expression in relation to clinicopathological, histopathological and biological factors

The frequency of LVD (we did not analyse the normal mucosa) at the periphery, the inner tumour area and the invasive margin was examined as shown in Figure 1. Among 138 primary rectal cancers, 18% ($n = 25$) of the hot spots were found at the periphery, 52% ($n = 72$) at the inner tumour area and 22% ($n = 30$) at the invasive margin; 8% ($n = 11$) of the cases were negative for D2-40. For the following analyses, the periphery was as one group and the inner tumour area, invasive margin and negative cases were combined as another group called the others on the basis of similarity of clinicopathological features.

In the whole series of the patients, a higher LVD at the periphery was related to better survival compared with the others ($P = 0.03$; Figure 2). Even in multivariate analysis, the prognostic significance still remained independent of the clinicopathological factors (TNM stage and the grade of differentiation) and biological factor (p53) ($P = 0.03$). Further, LVD at the periphery had a higher rate of negative p53 expression compared with the others ($P = 0.04$; Figure 3). LVD location was not related to the clinicopathological factors (TNM stage, differentiation grade, local recurrence and distant recurrence) or the histopathological factors (inflammatory infiltration and fibrosis) or the biological factor (apoptosis) ($P > 0.05$, data not shown).

No significance was found between LVD location and survival in the subgroups of non-RT ($P = 0.30$) and RT ($P = 0.50$).

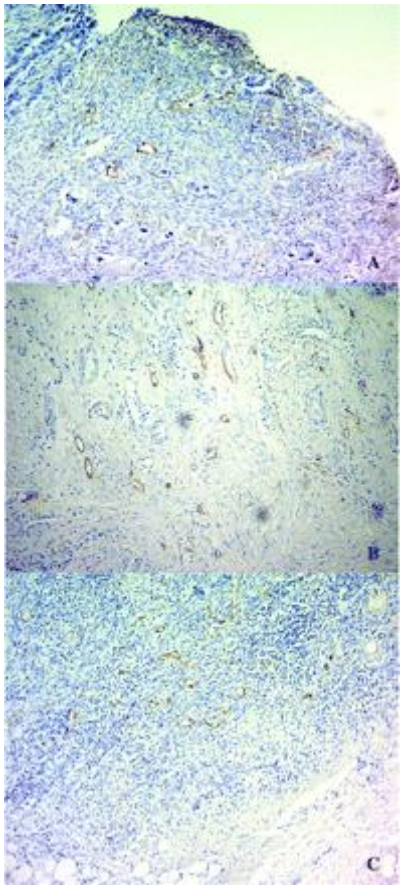

Figure 1: *Lymphatic vessel density at the periphery (A), inner tumour area (B) and invasive margin (C) in rectal cancer.*

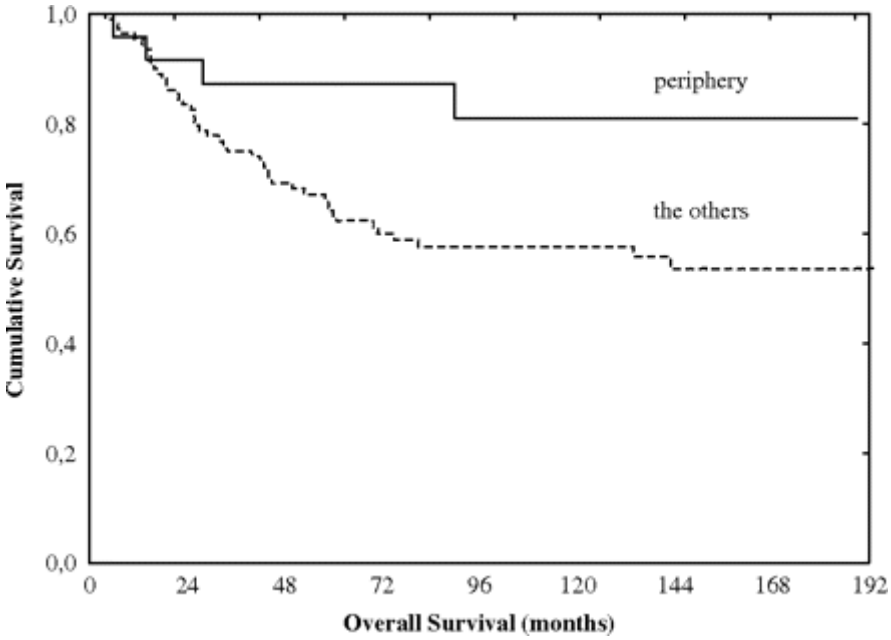

Figure 2: *Relationship between lymphatic vessel density location and overall survival in the entire group of the patients.*

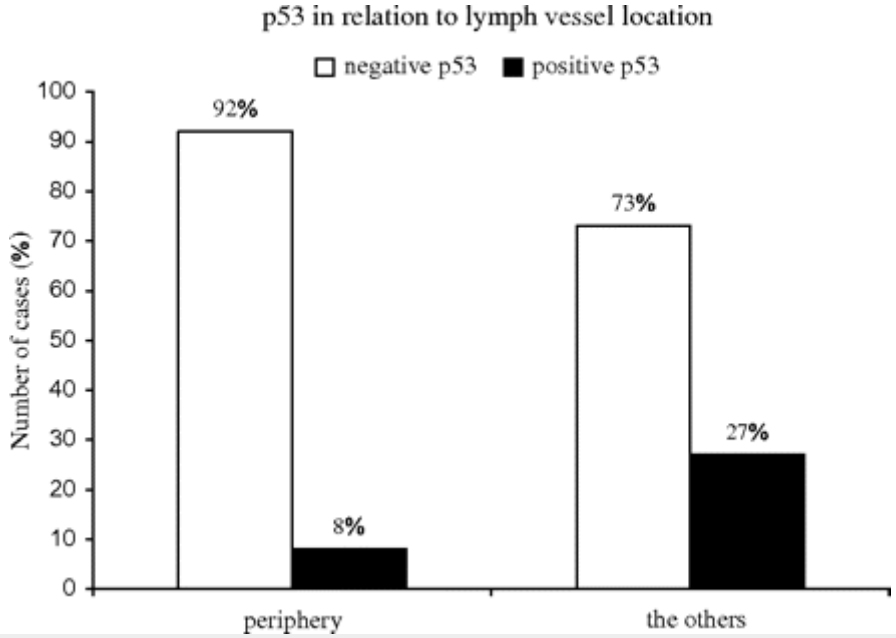

Figure 3: *p53 expression in relation to lymphatic vessel density location in rectal tumours with or without radiotherapy.*

In the non-RT group, a higher LVD at the periphery tended to be related to negative p53 expression ($P = 0.06$) but not in the RT group.

BVD and its relation to the location in tumours were examined (we did not analyse the normal mucosa) in the whole series of the patients, as well as in the subgroups of non-RT and RT. Among 140 tumours, 63% ($n = 88$) of the hot spots for BVD were located at the periphery of the tumour, 34% ($n = 47$) at the inner tumour area and 3% ($n = 5$) at the invasive margin (Figure 4), there were no negative cases for BVD. A high BVD at the periphery was related to worse survival in the whole series of the patients ($P = 0.02$) and in patients without RT ($P = 0.007$; Figure 5). In patients without RT, adjustment for the clinicopathological factors (TNM stage and the grade of differentiation) showed a trend towards significance ($P = 0.05$). There was no significant relationship to survival in patients with RT ($P = 0.65$).

In the non-RT subgroup, BVD at the periphery was related to TNM stage IIB–IV compared with TNM stage I–IIA (58% of 45 cases versus 31% of 29 cases, $P = 0.02$).

MVD of D2-40 and CD34 expression in relation to clinicopathological, histopathological and biological factors

The LVD and BVD in normal mucosa and tumour were analysed from the mean value of three hot spots in each specimen regardless of the location of the hot spots. The mean LVD (19.3 ± 14.1) and BVD (125.1 ± 54.5) in tumours were significantly higher than those in distant (LVD, 7.0 ± 4.2; BVD, 128.6 ± 45.1) or adjacent normal mucosa (LVD, 4.9 ± 3.9; BVD, 88.6 ± 41.9) ($P < 0.0001$; Table 3). Similar relationships were found between LVD/BVD in normal mucosa and tumours when the non-RT and RT group were analysed separately. There was no difference of the mean LVD between distant and adjacent normal

mucosa ($P < 0.05$, data not shown). The mean BVD decreased from distant normal mucosa to adjacent normal mucosa ($P = 0.001$; Table 3).

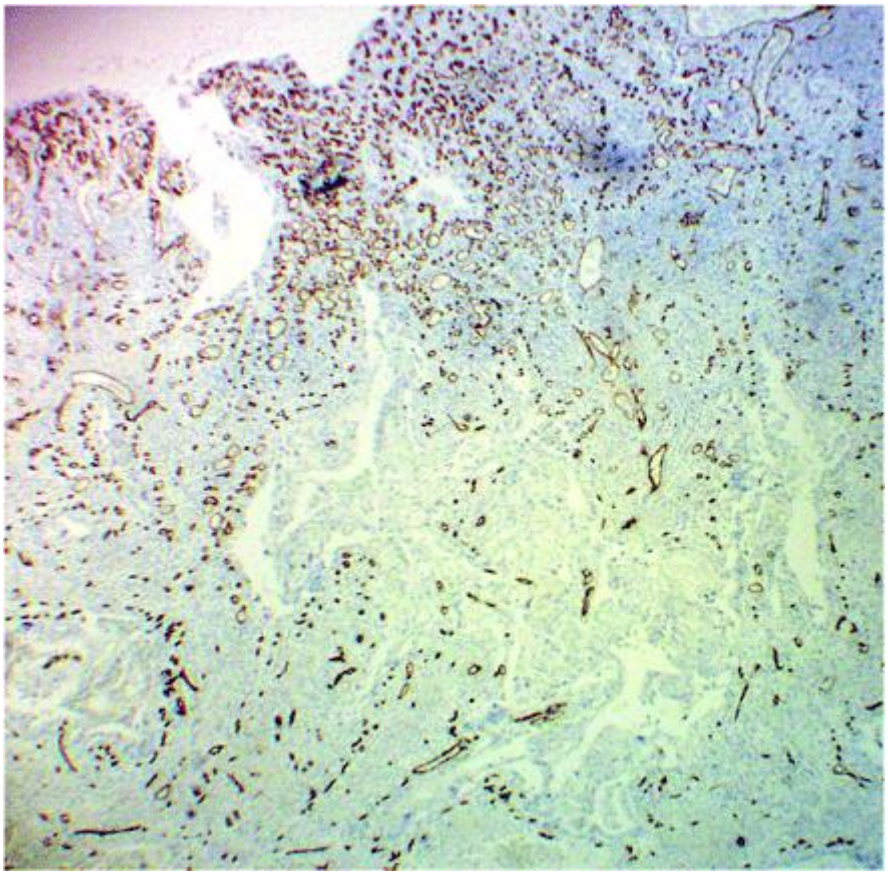

Figure 4: *Blood vessel density decreased from the periphery to the inner tumour area and invasive margin of the rectal cancer.*

We further examined relationships of the mean LVD and BVD with histopathological parameters (inflammatory infiltration and fibrosis) and biological factors (apoptosis and p53) on the basis of RT.

Tumours having negative p53 significantly increased LVD after RT compared with tumours having positive p53 ($P = 0.01$).

In the non-RT group, a high LVD was related to the clinicopathological factor (less distant recurrence, $P = 0.02$), histopathological factors (strong infiltration, $P = 0.03$ and more fibrosis $P = 0.03$) and the biological factors (less apoptosis, $P = 0.04$ and positive p53 expression, $P = 0.04$).

BVD was positively related to fibrosis in the whole group ($P = 0.005$) and in the RT group ($P = 0.002$) but not in the non-RT group ($P = 0.59$).

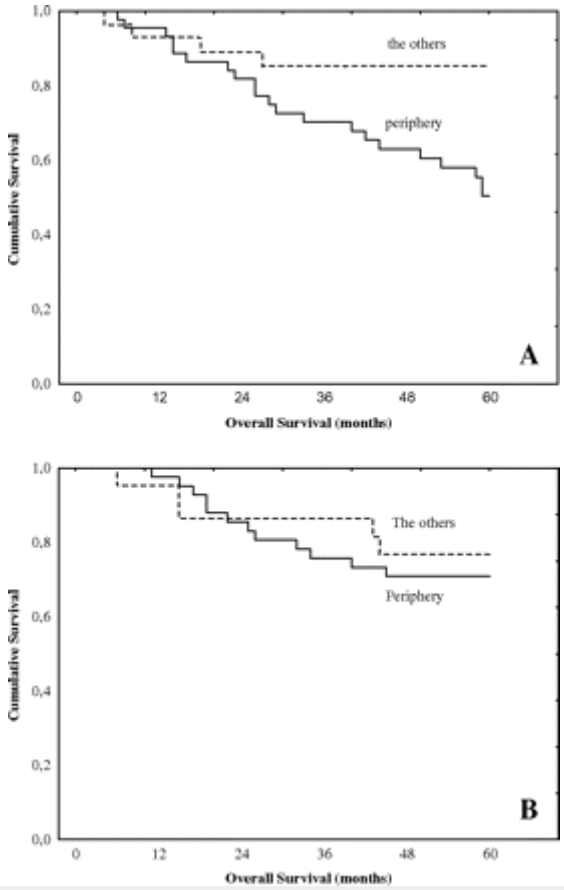

Figure 5: *Relationship between blood vessel density location and overall survival in the subgroups of the patients without radiotherapy (RT) (A) or with RT (B).*

Table 3: *LVD and BVD in normal mucosa and rectal cancer*

Characteristics		Distant normal mucosa	Adjacent normal mucosa	Cancer	*P**
LVD					
	Range	1.0–17.7	0.0–13.8	0.0–77.2	
	Mean ± SD	7.0 ± 4.2	4.9 ± 3.9	19.3 ± 14.1	<0.0001
	Median	7.0	4.3	18.5	
BVD					
	Range	63.7–227.0	2.8–203.7	23.5–286.5	
	Mean ± SD	128.6 ± 45.1	88.6 ± 41.9	125.1 ± 54.5	<0.0001
	Median	121.2	85.0	124.25	

**P* value for LVD or BVD difference in cancer versus distant/adjacent normal mucosa.
LVD, lymphatic vessel density; BVD, blood vessel density; SD, standard deviation.

Discussion

This is the first study of LVD location in rectal cancer patients who participated in a clinical trial of preoperative RT. In the whole series of the patients, LVD at the periphery of the tumour was related to better survival compared with LVD at the inner tumour area and the invasive margin. The prognostic significance still remained even after adjustment for TNM

stage, differentiation and p53 expression. The LVD location in the subgroups of non-RT ($n = 74$) and RT ($n = 66$) was analysed individually, but no statistical significance was found, probably due to a small number of the cases. A few have investigated the location of LVD in tumours and its relationship to clinical outcome [7, 9]. Among them, Maula et al. [9] studied 97 head and neck cancer patients who received preoperative RT and found a high density of PT related to a better outcome compared with vessels located IT. The positive relationship between LVD at the periphery and survival might be explained by a lower malignancy grade of tumour cell at the periphery compared with the invasive margin [18]. It was shown in cell-line studies that the invasive margin had an overexpression of VEGF-C, which dilates the lymphatic vessel lumen, making it easier for malignant cells to transit into the lymphatic circulation [17, 19]. The positive relationship between a high LVD at the periphery of the tumour and a better outcome may indicate that fewer metastases occur at the periphery of the tumour compared with the inner tumour area/invasive margin.

p53 are known to regulate DNA repair, cell cycle arrest and programmed cell death. Earlier, our study on the same series of the cases showed that negative (wild type) p53 expression was related to less local recurrence and a better outcome than positive p53 expression [15]. In the whole series of the patients, our present study on LVD location showed that tumour cells at the periphery had a higher expression of negative p53 compared with the others. This result strengthens our positive relationship between LVD location and survival. The periphery seems to have a high level of lymphatic vessels related to a better outcome and tumour cells with a high expression of negative p53 (wild type), which might make the tumour cells less aggressive. Together, the tumour cells and their surrounding lymphatic vessels are indicated to create an environment that makes it difficult for malignant cells to transit into the lymphatic circulation.

The relationship between MVD and survival has been studied extensively. It was shown that a high MVD was related to worse survival in many different types of cancers [1, 3]. In line with the previous results, we found a positive relationship between a high BVD at the periphery and a higher TNM stage (IIB–IV) in patients without RT.

The importance of angiogenesis and its response to RT has been recently raised. After RT, the survival for patients with BVD at the periphery tended to improve. This relationship was studied by one previous group on oropharyngeal cancer patients who showed that a decrease in MVD after RT correlated significantly with response to irradiation and overall survival [20]. The underlying mechanism of the increased survival of MVD at the periphery after RT might be explained by destructed blood vessels induced by RT which reduces the surface area for potential escape of tumour cells into the systemic circulation [10]. The best clinical effect of RT on tumour tissue is known to be received ~5 weeks after RT. In our study, the patients received preoperative RT and went through surgery within 1–13 days after RT. The interval between RT and surgery in our study might be too short to receive the optimal clinical effect by RT.

Inflammatory infiltration and fibrosis are known histological factors related to improved survival in colorectal cancer patients. Inflammatory infiltration kills tumour cells by lysis and fibrosis has a growth-limiting effect on tumour cells. In tumours without RT, others and we found a positive relationship between LVD, inflammatory infiltration and fibrosis [8, 21]. Inflammatory cells secret growth factors like VEGF-C ,-D and tumour necrosis factor-β that promotes proliferation of lymphatic vessels and stimulate fibroblasts to produce fibrosis [22, 23]. Inflammatory infiltration is indicated to induce both lymphangiogenesis and fibrosis in

tumours. In the present study, we found the association of high BVD and more fibrosis in the whole group and the RT group, but not in the non-RT group. Okudera et al. [21] showed a positive relationship between increased MVD and fibrosis in lung cancer. RT is indicated to induce the formation of TGF-β, which stimulates the production of fibrosis and increases MVD in normal and cancer human tissue [22].

In our previous study, we found a trend that after RT both necrosis and fibrosis were increased in p53-negative tumours [13, 15]. Here, we showed that LVD increased by RT in p53-negative tumours compared with p53-positive tumours, which makes us believe that p53 induced by RT in some way also affect LVD.

In summary, LVD at the periphery of the tumour was related to negative p53 and to a better outcome, while the degree of LVD/BVD was not related to survival. Our results may raise a notion that the location of the lymphatic vessels needs to be addressed on further cancer studies.

Funding

Swedish Cancer Foundation; Health Research Council in the south-east of Sweden.

Disclosure

The authors confirm that there is no conflict of interest or funding sources that might generate a conflict of interest.

References

1. Miyata Y, Kanda S, Ohba K, et al. *Lymphangiogenesis and angiogenesis in bladder cancer: prognostic implications and regulation by vascular endothelial growth factors-A, -C and –D. Clin Cancer Res 2006;12:800-806.*
2. Yonemura Y, Endo Y, Tabata K, et al. *Role of VEGF-C and VEGF-D in lymphangiogenesis in gastric cancer. Int J Clin Oncol 2005;10:318-327.*
3. Kuokourakis MI, Giatromanolaki A, Sivridis E, et al. *Cancer vascularisation: implications in radiotherapy? Int J Radiat Oncol Biol Phys 2000;48:545-553.*
4. Nakamura Y, Yasuoka H, Tsujimoto M, et al. *Importance of lymph vessels in gastric cancer: a prognostic indicator in general and a predictor for lymph node metastasis in early stage cancer. J Clin Pathol 2006;59:77-82.*
5. Parr C, Jiang WG. *Quantitative analysis of lymphangiogenic markers in human colorectal cancer. Int J Oncol 2003;23:533-539.*
6. Staton CA, Chetwood AS, Cameron IC, et al. *The angiogenic switch occurs at the adenoma stage of the adenoma carcinoma sequence in colorectal cancer. Gut 2007;56:1426-1432.*
7. Bono P, Wasenius VM, Heikkilä P, et al. *High LYVE-1-positive lymphatic vessel numbers are associated with poor outcome in breast cancer. Clin Cancer Res 2004;10:7144-7149.*
8. Kaneko I, Tanaka S, Oka S, et al. *Lympatic vessel density at the site of deepest penetration as a predictor of lymph node metastasis in submucosal colorectal cancer. Dis Colon Rectum 2006;50:13-21.*

9. Maula S, Luukkaa M, Grenman R, et al. *Intratumoral lymphatics are essential for the metastatic spread and prognosis in squamous cell carcinoma of the head and neck region. Cancer Res 2003;63:1920-1926.*
10. Coen I, Castermans K, Lammering G, et al. *Effects of radiotherapy and chemotherapy on angiogenesis and leukocyte infiltration in rectal cancer. Int J Radiat Oncol Biol Phys 2006;66:1219-1227.*
11. Jackowski S, Janusch M, Fiedler E, et al. *Radiogenic lymphangiogenesis in the skin. Am J Pathol 2007;171:338-345.*
12. *Swedish Rectal Cancer Trial: improved survival with preoperative radiotherapy in resectable cancer. N Engl J Med 1997;336:980-987.*
13. Knutsen A, Adell G, Sun X-F. *Inflammatory infiltration, fibrosis, necrosis and mucinous content in relation to clinicopathological and molecular factors in rectal cancers with or without preoperative radiotherapy. Oncol Rep 2006;16:321-327.*
14. Adell GC, Zang H, Evertsson S, et al. *Apoptosis in rectal cancer: prognosis and recurrence after preoperative radiotherapy. Cancer 2001;91:1870-1875.*
15. Adell GC, Sun X-F, Stål O, et al. *p53 status: an indicator for the effect of preoperative radiotherapy in rectal cancer. Radiother Oncol 1999;51:169-174.*
16. Vermeulen PB, Gasparini G, Fox SB, et al. *Quantification of angiogenesis in solid human tumors: an international consensus on the methodology and criteria of evaluation. Eur J Cancer 1996;32A:2474-2484.*
17. Padera T, Kadambi A, Tomaso E, et al. *Lymphatic metastasis in the absence of functional intratumor lymphatics. Science 2002;296:1883-1886.*
18. Palmqvist R, Oberg A, Bergström C, et al. *Systematic heterogeneity and prognostic significance of cell proliferation in colorectal cancer. Br J Cancer 1998;77:917-925.*
19. He Y, Rajantie I, Pajusola K, et al. *Vascular endothelial cell growth factor receptor 3-mediated activation of lymphatic endothelium is crucial for tumor cell entry and spread via lymphatic vessels. Cancer Res 2005;65:4739-4746.*
20. Lövey J, Lukits J, Remenár E, et al. *Antiangiogenic effects of radiotherapy but not initial microvessel density predict survival in operable oropharyngeal squamous cell carcinoma. Strahlenther Onkol 2006;182:149-156.*
21. Okudera K, Kamata Y, Takanashi S, et al. *Small adenocarcinoma of the lung: prognostic significance of central fibrosis chiefly because of its association with angiogenesis and lymphangiogenesis. Pathol Int 2006;56:494-502.*
22. Martin M, Lefaix J-L, Delanian S. *TGF-β1 and radiation fibrosis: a master switch and a specific therapeutic target? Int J Radiat Oncol Biol Phys 2000;47:277-290.*
23. Angeli V, Randolph G. *Inflammation, lymphatic function, and dendritic cell migration. Lymphat Res Biol 2006;4:217-228.*