Independent Prognostic Impact of Lymphatic Vessel Density and Presence of Low-Grade Lymphangiogenesis in Cutaneous Melanoma

Oddbjørn Straume, David G. Jackson, and Lars A. Akslen

Department of Pathology, The Gade Institute, University of Bergen, N-5021 Bergen, Norway [O. S. L. A. A.], and Medical Research Council Human Immunology Unit, Weatherall Institute of Molecular Medicine, John Radcliffe Hospital, Oxford OX3 9DS, United Kingdom [D. G. J.]

ABSTRACT

The aim of this study was to determine lymphatic vessel density (LVD) in a series of nodular melanoma and correlate the findings with the expression of several angiogenic factors, including vascular endothelial growth factor-C, basic fibroblast growth factor (bFGF), patient survival, and clinico-pathologic data. Patients with nodular melanoma and complete follow-up information were included. Lymphatic vessels were immunostained with the LYVE-1 and Podoplanin antibodies, and LVD was evaluated in both intra- and peri-tumoral areas. Median LVD was 6.3 and 12.5 vessels/mm² in intra- and peri-tumoral areas. Coexpression of LYVE-1 and Ki-67/MIB-1 in lymphatic endothelial cells within the tumor was demonstrated, indicating active but low-grade lymphangiogenesis. Increased LVD was significantly associated with localization on the extremities (P = 0.005), decreased tumor thickness (P = 0.036), absence of vascular invasion (P = 0.004), brisk lymphocytic infiltration (P = 0.018), low proliferative rate by Ki-67 (P = 0.011), increased bFGF expression in tumor cells (P = 0.01) as well as in endothelial cells (P = 0.008), and decreased tumor cell expression of Ephrin-A1 (P = 0.009). Decreased LVD in intra-tumoral areas and LVDpt both predicted improved survival rates in multivariate analyses (for LVDpt, Hazard ratio: 2.1, P = 0.009). We found that decreased LVD was present in thicker and more proliferative tumors (Ki-67) and that increased LVD was significantly associated with improved patient survival in multivariate analysis. In addition, our data suggest the presence of low-grade intra-tumoral lymphangiogenesis in melanoma and a stimulating role of bFGF in lymphangiogenesis.

INTRODUCTION

Lymphangiogenesis has been recently considered important for metastatic spread and prognosis of malignant tumors (1-4). The significance of lymphatic vessels has also been focused by the identification of VEGF-C (5) and VEGF-D (6) as stimulators of lymphatic endothelial proliferation, the VEGF receptor-3 (Flt-4; Ref. 7) as a specific receptor in normal adult tissues, and the finding of markers for lymphatic endothelium (8-10). Experimental studies strongly suggest that intra-tumoral lymphangiogenesis does occur in the presence of VEGF-C (3, 11, 12) and VEGF-D (2). Evidence has also been presented that these growth factors may increase the development of lymph node metastases, probably through their effects on lymphatic vessels (2, 3, 11, 13). Whether de novo lymphangiogenesis is necessary for lymphatic spread of naturally occurring cancers is not known, because tumor cell invasion of pre-existing lymphatics at the tumor margins might also occur (14-17).

The role of lymphangiogenesis for growth and spread of cutaneous melanoma is unclear. Previous studies have concluded that lymphatic vessel formation does not occur in these tumors (18), because there was no increase in the number of intra-tumoral lymphatic vessels from thin to thick melanomas, in contrast to what was found for blood vessels. It is, however, well established that cutaneous melanomas show frequent spread by the lymphatic route (19), but it is not known whether this process requires active intra-tumoral lymphangiogenesis. The importance of VEGF-C-induced metastasis in melanomas has been suggested recently in animal studies (20), although the relationship with LVD remains to be elucidated in human melanoma tissue.

On this background, the aim of our study was to determine the intra- and peri-tumoral LVD using the LYVE-1 ad Podoplanin antibodies (8) in a large series of VGP melanoma and correlate the findings with the expression of several angiogenic factors, including VEGF-C and bFGF, patient survival, and clinico-pathologic data.

MATERIALS AND METHODS

Patients. Of all the melanomas occurring in Hordaland County (10% of the Norwegian population) during 1981-97, 97.5% were diagnosed at the Department of Pathology, The Gade Institute, Haukeland University Hospital. There were no

Received 3/7/02; revised 8/12/02; accepted 8/19/02.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 Supported by Norwegian Cancer Society Grant D-94070.
2 To whom requests for reprints should be addressed, at Department of Pathology, The Gade Institute, Haukeland University Hospital, N-5021 Bergen, Norway. Phone: 47 55 97 31 82; Fax: 47 55 97 31 58; E-mail: lars.akslen@gades.uib.no.

The abbreviations used are: VEGF, vascular endothelial growth factor; LVD, lymphatic vessel density; VGP, vertical growth phase; IHC, immunohistochemistry; MVD, microvessel density; bFGF, basic fibroblast growth factor; LVDit, intra-tumoral lymphatic vessel density; HR, hazard ratio; Lratio, likelihood ratio; LVDpt, peri-tumoral lymphatic vessel density; HPF, high powered field.
differences in sex, anatomical site, or stage between these cases and the 2.5% with a diagnosis from other laboratories, although the latter patients were 6 years younger (median age). The aim of this study was to focus on the aggressive subgroup consisting of nodular melanomas, which are all VGP melanomas. After microscopic review of all cases diagnosed and recorded as malignant melanoma of the nodular type or not otherwise specified during this period, 202 cases were included. The presence of a VGP and the lack of a radial growth phase, i.e., adjacent in situ or micro-invasive component, were used as inclusion criteria (21), and only primary tumors were included after careful examination of all slides. There was no history of familial occurrence. Complete information on patient survival, time, and cause of death were available in all 202 cases. Last date of follow-up (with respect to recurrences) was not carried out in 14 (mostly older) patients, and 21 patients were not treated with complete local excision. Thus, recurrence-free time could be studied in 167 patients.

**IIIC.** IHC was performed on formalin-fixed and paraffin-embedded archival tissue. Sections (5 μm) were dewaxed in xylene, and epitope retrieval was performed by microwaving for 3 × 5 min in 0.1 M Tris-HCl (pH 9.0) with 2 mM EDTA at 500 W. The rabbit polyclonal LYVE-1 antibody (125 μg/ml; Ref. 8) was diluted 1:100 and incubated overnight at room temperature. Immunoperoxidase staining was carried out using the DAKO Envision Kit (DAKO, Copenhagen, Denmark) and 3-amino-9-ethylcarbazole-peroxidase as substrate before counterstaining with Harris hematoxylin. Omission of the primary antibody was used as a negative control.

Serial sections from 25 randomly selected cases were stained with Podoplanin (9) for comparison with LYVE-1. Sections were deparaffinized and microwaved in citric buffer (pH 6.0) for 3 × 5 min. The rabbit polyclonal antibody Podoplanin was diluted 1:400, incubated for 2 h at room temperature (9), and stained with the DAKO Envision Kit as described above.

To demonstrate the presence of proliferating lymphatic endothelial cells, 10 random cases with high LVDt counts (above 75 percentile) were selected for LYVE-1/Ki-67 double staining. Tissues were pretreated as described above and incubated with LYVE-1 (1:50) and Ki67/MIB-1 (Oncogene, Boston, MA) for 2 h at room temperature. LYVE-1 was detected as described above, whereas Ki-67 staining was detected by a biotinylated goat antimouse secondary antibody 1:50 (E0433; DAKO) and a streptavidin alcalic phosphatase complex (K0391; DAKO) with Fast Blue as chromogen.

After microwave antigen retrieval (10 min at 750 W and 4 × 5 min at 500 W), the sections were incubated for 1 h (room temperature) with the polyclonal Ki-67 antibody (code no. A-047; DAKO) and diluted 1:50. The staining procedures and evaluation of other markers included have been described previously (22–24), and the results of these are included for comparison (see “Results”). The analyses of some of the markers (Ephrin-A1, EphA2, interleukin-8, VEGF-C, and FLT-4; examined in 155 cases) were performed on tissue microarray sections as described (24).

**Evaluation of IHC.** LVD and MVD were assessed as described previously for MVD (23): 175 cases were evaluable for LVDt, whereas 27 tumors were recorded as missing attributable to insufficient tumor material left in the tissue blocks; 169 cases could be evaluated for LVDpt. Briefly, the sections were scanned at low magnifications (×25 and ×100) to identify the areas of the tumors with the highest amount of lymphatic vessels (“hot spots”), similar to Weidner et al. (25) and Birner et al. (4). Intra-tumoral hot spots were most frequently found in the upper third of the tumor. Within these areas, five fields at ×400 magnification (HPF, 0.16 mm²/field) were examined, and the mean value of these fields as well as the maximum value were calculated. Vessels more than one-half HPF (×400) away from (below) the invasive front, or vessels close (<0.5 HPF) to ulcerated areas, were not counted. Any endothelial cell or cell cluster, highlighted by LYVE-1 reactivity and clearly separate from adjacent vessels, tumor cells, and connective tissue elements, was regarded as a distinct countable vessel (25). Specific LVD counts were established for LVDt and LVDpt areas for each case, and the counts are given as vessels per millimeter squared. The predominant appearance of peri-tumoral lymphatic vessels was also recorded (compressed and/or angulated versus rounded/dilated).

MVD was evaluated similarly to LVD, and 10 consecutive fields were counted in the hot spots using Factor-VIII staining. In addition, specific MVD counts were established for the central tumor areas (intra-tumoral MVD) as reported (23). The pattern of tumor-infiltrating lymphocytes was recorded as absent/nonbrisk and brisk according to the criteria of Clark et al. (26). Ki-67 staining was assessed according to the approach of Weidner et al. (27). Briefly, tumors were scanned at low magnification (×40 and ×100) to identify areas of most intense nuclear staining, and these hot spots were most often found in the periphery of the tumor, i.e., near the invasive front (tumor basis) or near the epidermis (22). The percentage of immunoreactive tumor cell nuclei (proliferative rate) was then calculated by counting ≥500 cells at ×1000 within the selected areas.

**Statistics.** Analyses were performed using the statistical package SPSS ver. 10.1 (28). Associations between different categorical variables were assessed by Pearson’s χ² test. Continuous variables not following the normal distribution were compared between two or more groups using the Mann-Whitney U or Kruskal-Wallis H tests or Spearman’s rank correlation by two continuous variables. Univariate analyses of time to death caused by malignant melanoma or time to recurrence (recurrence-free survival) were performed using the product limit procedure (Kaplan-Meier method), with date of histological diagnosis as the starting point. Patients who died of other causes were censored at the time of death. Differences between categories were tested by the Log-rank test. The influence of co- variates on patient survival and recurrence-free survival was analyzed by the proportional hazards method (29), including all variables with a P ≤ 0.15 in univariate analyses, and tested by the Lratio test. Model assumptions were tested by log-minus-log plots, and significant variables were tested for interactions. Estimated HR, 95% confidence interval for HR, and Ps are given in the tables.
RESULTS

LVD. Staining of lymphatic endothelial cells was strong and distinct when present (Fig. 1), and endothelial cells in vessels containing RBCs were negative. Weak cytoplasmic positivity was occasionally observed in tumor-associated macrophages, as well as in some tumor cells, but this was not significant in most tumors. In the majority of cases, lymphatic vessels appeared to be angulated or collapsed both intra- and peri-tumoral, although predominantly dilated (rounded) peri-tumoral lymphatics were recorded in 9% of the cases.

The results are given as the mean count of five HPFs (vessels per millimeter squared) for LVDit and LVDpt, separately; the mean counts and the maximum counts (see “Materials and Methods”) showed similar associations with clinico-pathologic variables.

Distinct staining of intra- and peri-tumoral lymphatic vessels was identified in 57 and 82% of the cases, respectively. Twenty-eight cases (17%) were negative for both. Dermal lymphatics in the surrounding tissue was used as a positive internal control in otherwise negative cases. Median LVDit was 6.3 vessels/mm² (mean, 11.5; SD, 15.4; range, 0–93), and the values were distributed as illustrated in Fig. 2. The median LVDpt was 12.5 vessels/mm² (mean, 14.3; SD, 12.4; range, 0–75). LVDit and LVDpt were significantly associated (Spearman’s rank correlation, \( P < 0.0001 \)). In 75 cases (44% of all), counts for LVDpt were higher than for LVDit, whereas in 19% of all cases, LVDit counts were highest.

Table 1 summarizes the significant associations between LVDit or LVDpt and anatomical site, Clark’s level of invasion, vascular invasion, lymphocytic infiltration, bFGF expression in tumor cells and in endothelial cells, as well as expression of ephrin-A1. Regarding continuous variables, increased LVDit was significantly associated with younger age (Spearman’s rank correlation, \( P = 0.034 \)) and increased MVD as well as intra-tumoral MVD by Factor-VIII (Spearman’s rank correlation, \( P = 0.04 \) and 0.05, respectively). Similarly, increased LVDpt was associated with younger age (Spearman’s rank correlation, \( P < 0.001 \)) and decreased tumor thickness (Spearman’s rank correlation, \( P = 0.036 \)) but not significantly with MVD (Spearman’s rank correlation, \( P = 0.13 \)). Furthermore, increased LVDpt was associated with tumor cell proliferation (Ki-67) below median value (Mann-Whitney, \( P = 0.01 \)), whereas LVDit was not. Cases with absent/weak p16 protein expression had a lower mean LVDit count than the others, 9.8 versus 12.9 (Mann-Whitney, \( P = 0.026 \)). For LVDpt, there was no significant association with p16 expression.

There were no significant associations between LVDit or LVDpt and the following markers were analyzed: (a) ulceration; (b) presence of lymph node metastases at diagnosis; (c) endothelial cell expression of Flt-1 and KDR; (d) tumor cell expression of VEGF; (e) Flt-1; (f) KDR; (g) VEGF-C; (h) Flt-4; (i) EphA2; (j) interleukin-8; or (k) thrombospondin-1 in the tumor stroma.

In the double-stained tumors, coexpression of LYVE-1 and Ki67/MIB-1 was observed in both intra- and peri-tumoral lymphatics in 5 of the 10 cases analyzed (Fig. 1), although the majority of nuclei in lymphatic endothelial cells were negative.

Sixteen cases (9%) were regarded to have predominantly

---

**Fig. 1** Immunohistochemical staining of lymphatic vessels by the LYVE-1 antibody in nodular melanoma (A); blood vessels are negative. B, serial section of the same case stained with Podoplanin. Double staining for LYVE-1 (red) and Ki-67/MIB-1 (blue) of intra-tumoral (C) and peri-tumoral (D) lymphatic vessels; positive staining for Ki-67/MIB-1 is seen in nuclei of lymphatic endothelial cells (black arrows). Arrowheads, blood vessels; E, epidermis; T, tumor.
dilated peri-tumoral lymphatics. Dilated lymphatic vessels showed a significant association with increased LVDpt (Mann-Whitney test, \( P < 0.004 \)) and LVDit (Mann-Whitney test, \( P < 0.05 \)). Furthermore, dilation was associated with tumor cell expression of bFGF (\( P < 0.007 \)).

Serial sections, stained with Podoplanin and LYVE-1, were closely studied to compare the staining patterns. The two antibodies had a strikingly similar staining pattern as illustrated in Fig. 1. In contrast to LYVE-1, Podoplanin also showed some staining of the basal epidermal cells in a majority of the cases. Vessels containing RBCs were found to be negative for both lymphatic markers. Median LVDit and LVDpt by LYVE-1 were 6.3 and 12.5 vessels/mm², compared with 6.9 and 18.7 vessels/mm² by Podoplanin, respectively. The lymphatic vessel counts were significantly correlated between the two markers (Spearman’s \( \rho \) correlation coefficient = 0.77; \( P < 0.0001 \) and \( \rho = 0.74 \) for LVDit, \( P < 0.0001 \) for LVDpt).

Patient Survival. The patients were divided into two groups by the median value of LVDit and LVDpt. Five-year survival in cases with high LVDit was 74%, compared with 53% in cases with low LVDit (Log-rank test, \( P = 0.003 \)). The values were 77 and 49% in cases with high and low LVDpt, respectively (Log-rank test, \( P < 0.0001 \); Fig. 3). LVDit did not significantly predict recurrence-free survival (Log-rank test, \( P = 0.09 \)), whereas 5-year, recurrence-free survival was 59% in cases with high LVDpt, compared with 11% in cases with low LVDpt (Log-rank test, \( P < 0.0001 \); Fig. 3).

In multivariate analysis of patient survival, LVDpt was an independent prognostic variable (HR, 2.1; \( P = 0.009 \)) when included along with known prognostic factors like tumor thickness, Clark’s level of invasion, vascular invasion, tumor ulceration, tumor cell proliferation by Ki-67, and MVD. Similarly, LVDit was also significant in the multivariate model of patient survival (HR, 1.9; \( P = 0.017 \)). The final multivariate model for LVDpt is given in Table 2.

DISCUSSION

Recently, the importance of lymphangiogenesis for malignant tumors has been reported (2, 3, 30, 31), and lymphangiogenic factors (VEGF-C, VEGF-D, and VEGF receptor-3) were shown to play a regulatory role (2, 3, 31). However, these experimental studies were performed in mouse models (2, 3), and the importance of lymphatics in human cancer remains to be clarified (14–16).

<table>
<thead>
<tr>
<th>No. of patients</th>
<th>LVDit</th>
<th>LVDpt</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anatomic site</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Head/neck</td>
<td>43</td>
<td>12.0</td>
</tr>
<tr>
<td>Trunk</td>
<td>46</td>
<td>8.8</td>
</tr>
<tr>
<td>Upper extremity</td>
<td>35</td>
<td>13.0</td>
</tr>
<tr>
<td>Lower extremity</td>
<td>50</td>
<td>12.7</td>
</tr>
<tr>
<td>Clark’s level of invasion</td>
<td></td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>18</td>
<td>15.3</td>
</tr>
<tr>
<td>IV</td>
<td>124</td>
<td>10.7</td>
</tr>
<tr>
<td>V</td>
<td>33</td>
<td>12.5</td>
</tr>
<tr>
<td>Vascular invasion</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absent</td>
<td>138</td>
<td>11.2</td>
</tr>
<tr>
<td>Present</td>
<td>37</td>
<td>12.7</td>
</tr>
<tr>
<td>Lymphocytic infiltration</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absent/nonbrisk</td>
<td>99</td>
<td>9.8</td>
</tr>
<tr>
<td>Brisk</td>
<td>75</td>
<td>13.7</td>
</tr>
<tr>
<td>bFGF in tumor cells</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absent/weak</td>
<td>97</td>
<td>9.7</td>
</tr>
<tr>
<td>Moderate/strong</td>
<td>76</td>
<td>14.0</td>
</tr>
<tr>
<td>bFGF in endothelial cells</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absent</td>
<td>37</td>
<td>9.4</td>
</tr>
<tr>
<td>Present</td>
<td>136</td>
<td>12.1</td>
</tr>
<tr>
<td>Ephrin-A1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weak/moderate</td>
<td>122</td>
<td>12.7</td>
</tr>
<tr>
<td>Strong</td>
<td>23</td>
<td>11.1</td>
</tr>
</tbody>
</table>

<sup>a</sup> Mann-Whitney \( U \) test, when otherwise not specified.

<sup>b</sup> Kruskal-Wallis test.

<sup>c</sup> Staining index 0–1.

<sup>d</sup> Staining index < 9.

Table 1 LVD (mean vessel counts per millimeter squared) related to standard clinico-pathologic variables as well as angiogenesis regulators in 175 patients with VGP melanoma, specified for LVDit and LVDpt locations.

Fig. 2 Histogram showing the distribution of cases according to the intra-tumoral (A) and peri-tumoral (B) LVD per millimeter squared.
Studies of lymphangiogenesis and LVD rely on specific lymph-endothelial markers, and this has been a matter of discussion. LYVE-1 is in most instances specific for lymphatics, although expression of the receptor on liver and spleen sinusoids may complicate its use in these particular tissues. In skin and melanomas, as shown in our present study, LYVE-1 stains only lymphatics and was negative in vessels with RBCs. Moreover, the identity of the LYVE-1-positive vessels as lymphatics was further confirmed using another lymphatic marker Podoplanin (9), which revealed a similar staining pattern in serial sections from a random subgroup of cases, validating our LVD counts as a reliable estimate of lymphatic vessel numbers.

In our study, distinct staining of intra- and peri-tumoral lymphatic vessels was identified in 57 and 82% of cases, respectively. On average, the LVD was two times higher at the periphery of the melanomas, when compared with more central areas of each tumor. Intra-tumoral lymphatic vessels might represent an extension or recruitment of dermal lymphatics, which may be activated for growth or migration. However, our present results are also consistent with active but low-grade lymphangiogenesis in certain tumor subgroups, indicated by coexpression of LYVE-1 and Ki-67/MIB-1 in some intra- and peri-tumoral lymphatic vessels, supporting what others have suggested. Previously, the existence of true intra-tumoral lymphangiogenesis has been questioned.

We found that an increased LVD within the tumor as well as in the peri-tumoral areas was significantly associated with improved survival. To our knowledge, only one survival study of lymphatic vessels, using a different marker, has been published. Our findings of increased LVD as an independent and positive prognostic factor in VGP melanoma support the data of Birner et al. on cervical cancer. On the basis of recent animal studies, the opposite relationship might have been expected. One explanation for our findings might be that, for an immunogenic tumor like melanomas, the presence of a large and functional lymphatic network might provide an increased T cell-mediated immune response to tumor cells. Alternatively, large and aggressive melanomas might compress and destroy the lymphatics and possibly make them less detectable by IHC, consistent with our findings of decreased LVD in thicker tumors with high proliferative rate assessed by Ki-67 immunostaining and with similar findings in breast carcinomas. This process could in part be mediated by the activity of matrix metalloproteinases, which is

### Table 2

<table>
<thead>
<tr>
<th>Category</th>
<th>n</th>
<th>HR</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Level of invasion</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>II, III, IV (Clark)</td>
<td>135</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>V</td>
<td>31</td>
<td>2.3</td>
<td>0.01</td>
</tr>
<tr>
<td>Vascular invasion</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absent</td>
<td>131</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Present</td>
<td>35</td>
<td>2.4</td>
<td>0.003</td>
</tr>
<tr>
<td>Ki-67 expression</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤16%</td>
<td>39</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>&gt;16%</td>
<td>127</td>
<td>3.2</td>
<td>0.009</td>
</tr>
<tr>
<td>MVD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>110</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>High</td>
<td>56</td>
<td>1.8</td>
<td>0.04</td>
</tr>
<tr>
<td>LVDpt</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>74</td>
<td>2.1</td>
<td></td>
</tr>
<tr>
<td>High</td>
<td>92</td>
<td>1</td>
<td>0.009</td>
</tr>
</tbody>
</table>

* L ratio test.
* Lower quartile.
* 67%.
* Median value of LVDpt.

Fig. 3 **A**, survival curve according to the Kaplan-Meier method by peri-tumoral LVD in nodular melanoma with death caused by melanoma as end point. **B**, recurrence-free survival by peri-tumoral LVD.
reported to be associated with melanoma progression and survival (35), although independent of tumor thickness and level of coetaneous invasion (36).

The number of LYVE-1-positive lymphatic vessels was significantly decreased in thicker tumors with advanced Clark's level of coetaneous invasion and increased number of proliferating (Ki-67) tumor cells. More lymphatics are generally present near the papillary dermis than in the deeper reticular dermis (18). We found that peri-tumoral LVD was highest at the extremities, and because these lesions were significantly thinner, some of the difference in LVD according to anatomical site might be explained by tumor thickness. Still, LVD was an independent prognostic factor in the final multivariate model along with anatomical site, strongly supporting an individual role of LVD for patient prognosis.

Among the angiogenic factors tested, only the expression of bFGF, in both tumor cells and tumor-associated endothelium, was significantly associated with increased LVD. Tumor cell expression of bFGF was especially associated with the presence of peri-tumoral lymphatics and also with dilation of these. This relationship is supported by recent experimental findings that proliferation, migration, and invasion of lymphatic endothelial cells are stimulated by bFGF (37). A similar association was found recently between bFGF expression and melanoma angiogenesis by Factor-VIII-positive microvessels (24). Surprisingly, we found no association between LVD and the expression of VEGF-C or VEGF-A, despite evidence that the former can induce lymphangiogenesis within xenotransplanted tumors in mice (3, 31). Thus, the regulation of lymphatic vessel formation might be more complex and include networks of interacting growth factors, including bFGF, as is the case for blood vessel angiogenesis (24, 38).

In conclusion, a reduction of LYVE-1-positive lymphatic vessels was found in melanomas with greatest vertical thickness and tumor proliferation as assessed by Ki-67 immunostaining, and increased LVD was associated with improved survival in multivariate analysis. In addition, our data suggest a stimulatory role of bFGF in melanoma lymphangiogenesis.

ACKNOWLEDGMENTS

We thank Gerd Lilian Hallseth and Bendik Nordanger for excellent technical assistance and Dr. Donscho Kerjaschki, University of Vienna-Allgemeines Krankenhaus, Vienna, for the Podoplanin antibody.

REFERENCES


Independent Prognostic Impact of Lymphatic Vessel Density and Presence of Low-Grade Lymphangiogenesis in Cutaneous Melanoma

Oddbjørn Straume, David G. Jackson and Lars A. Akslen


Updated version  Access the most recent version of this article at:  
http://clincancerres.aacrjournals.org/content/9/1/250

Cited articles  
This article cites 35 articles, 12 of which you can access for free at:  
http://clincancerres.aacrjournals.org/content/9/1/250.full#ref-list-1

Citing articles  
This article has been cited by 21 HighWire-hosted articles. Access the articles at:  
http://clincancerres.aacrjournals.org/content/9/1/250.full#related-urls

E-mail alerts  
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions  
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions  
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.