Intratumoral Lymphatics and Lymph Node Metastases in Papillary Thyroid Carcinoma

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Objective: To examine the relationship between lymphatic vessel density and clinical and pathological variables in patients with well-differentiated papillary thyroid carcinoma.

Subjects: Clinical information was retrieved on 109 previously untreated patients with well-differentiated papillary thyroid carcinoma treated with total thyroidectomy and postoperative iodine I 131 ablation. Median follow-up was 38 months.

Design: Archived tissue specimens were sectioned and stained with hematoxylin-eosin and anti–LYVE-1 antibody, a highly specific marker for lymphatic endothelium. The size of the tumor and its multifocality were noted and lymphatic vessel density was measured by means of Chalkley point counting.

Results: Numerous intratumoral lymphatics were seen in papillary thyroid carcinoma. There was a highly significant association between the presence of intratumoral lymphatics and the presence of neck node metastases (P<.001). There was also a significant association with male sex (P=.03) and the presence of multifocal disease (P=.05). The presence of intratumoral lymphatics remained significantly associated with the presence of nodal metastases at presentation (P=.003) on multivariate analysis. Intratumoral lymphatics were not a significant predictor of tumor recurrence (P=.42, log-rank test).

Conclusions: The development of intratumoral lymphatics in well-differentiated papillary thyroid carcinoma appears to be associated with the spread of tumor to regional lymph nodes. The antimetastatic potential of targeting these lymphatics may be of potential therapeutic benefit in the future.


APILLARY THYROID carcinoma frequently metastasizes to regional lymph nodes. The prognostic significance of lymph node metastases at the time of presentation is controversial, with some authors finding it to be a significant predictor of recurrence and survival and others not. A matched-pair analysis from Memorial Sloan-Kettering Cancer Center, New York, NY, published in 1996 suggested that the presence of neck node metastases had a significant impact on recurrence only in patients older than 45 years. The mechanism by which tumors enter the lymphatics and spread to regional lymph nodes is unknown. It is assumed that tumor cells gain access to the lymphatics either by the proliferation and invasion of new lymphatics into and around the developing tumor or by cells invading preexisting lymphatics adjacent to the tumor. Historically, pathological studies have failed to detect lymphatics within tumors, leading to the assumption that intratumoral lymphatics do not exist. However, the recent demonstration of intratumoral lymphatic networks in a mouse model and in human tumors suggests that tumors do possess an intrinsic lymphatic supply and that intratumoral vessels can proliferate despite the high interstitial pressure within the tumor mass.

Studies of tumor lymphangiogenesis have been hampered by a lack of specific markers for lymphatic endothelium. Earlier investigations have relied on vessel morphologic characteristics under electron and light microscopy, 5′-nucleotidase enzyme histochemistry, or differential staining of lymphatics with CD31 and blood vessels with PAL-E. It was originally thought that VEGFR-3, a receptor for the lymphangiogenic factor vascular endothelial growth factor (VEGF) C, could be a specific marker for lymphatics, as its expression is restricted to the lymphatic endothelium in adult tissue, but studies have found it expressed in tumor blood vessels, thus limiting its usefulness for the assessment of tumor lymphatic vessel density (LVD).

Recently, 3 new genes have been reported that appear to be specific to lymphatic endothelial cells: (1) Prox 1, a homeobox...
gene expressed in a subpopulation of endothelial cells that gives rise to the lymphatic system, which is required for lymphatic development\(^1\), (2) podoplanin, an integral membrane protein originally identified in glomerular epithelial cells\(^2\); and (3) LYVE-1, a receptor for the extracellular matrix glycosaminoglycan hyaluronan.\(^3\)

The LYVE-1 is a specific marker for lymphatics in many normal tissues\(^4\) and maintains its specificity in tumor tissue.\(^5\) The precise function of the LYVE-1 receptor is unclear but may include the sequestration and uptake of hyaluronan from the lymph fluid or hyaluronan-mediated adhesion and migration of cells along the lymphatic endothelial wall.\(^6\)

The aims of this study were to investigate the location and morphologic characteristics of tumor lymphatics in patients with papillary thyroid carcinoma and to examine the relationship between LVD and clinical and pathological variables, particularly spread to regional lymph nodes.

### METHODS

One hundred nine previously untreated patients with well-differentiated papillary thyroid carcinoma, treated between 1990 and 1998, were identified from the Mount Sinai Hospital database. All patients underwent total thyroidectomy and selective neck dissection if metastatic nodal disease was palpable either preoperatively or intraoperatively. All tumors were examined by a single pathologist (S.L.A.), with thorough documentation of tumor size and multifocality as well as the presence of lymph node metastases. Patients with extrathyroidal extension or distant metastases were excluded so that the association between LVD and tumor spread to regional lymph nodes could be determined independent of these poor prognostic factors. Each patient had corresponding archived paraffin-embedded tissue specimens available. Patients underwent iodine I 131 ablation 3 months postoperatively and were placed on a regimen of a suppressive dose of thyroxine sodium. They were followed up clinically and with yearly serum thyroglobulin levels to detect recurrence. Clinical data were collected on the patients’ age, sex, presence or absence of nodal metastases, recurrence, location of recurrence, and time to recurrence or length of follow-up.

Four-micrometer sections of formalin-fixed, paraffin-embedded tissue were cut onto silanized glass slides. They were cleared of paraffin in xylene and rehydrated through graded alcohol baths. Sections were incubated in 0.03% hydrogen peroxide for 15 minutes to inactivate endogenous peroxidases and then pressure-cooked for 3 minutes in 0.1M citrate buffer, pH 6.0, and blocked in 10% normal human serum for 15 minutes. Slides were then incubated (30 minutes) with 1:100 rabbit polyclonal anti-LYVE-1 antibody\(^6\) in Tris-buffered saline and 5% normal human serum, rinsed twice in Tris-buffered saline and then developed using the immunostaining system HRP Envision System (DAKO, Carpinteria, Calif). Slides were counterstained with hematoxylin (Sigma Diagnostics, St Louis, Mo) and mounted with the coverslip mounting glue Aquamount (Lerner Laboratories, Pittsburgh, Pa). The LVD was determined in tumor vessel “hot spots” by means of a Chalkley point-counting grid at high power (\(\times 250\)) by 2 observers as described.\(^7\) The mean of the vessel counts in 3 hot spots per section was recorded.

The patient’s age, tumor size, and LVD were divided into 2 categories at the median value. The association between intratumoral LVD and sex, age, tumor size, multifocality, and nodal status at presentation were examined by the Pearson chi-squared test in a univariate model. Multivariate analysis was carried out with a bivariate logistic regression model, using a forward variable selection technique (entry \(P<.05\), removal \(P>.1\)), to identify which of the variables were independently associated with the presence of intratumoral lymphatics. Disease-free survival curves were estimated by the Kaplan-Meier method and compared with the log-rank test. All statistical analysis was done with SPSS software version 10.0 (SPSS Inc, Chicago, Ill).

### RESULTS

Numerous large, LYVE-1–positive, irregularly shaped, thin-walled lymphatics were identified in sections of normal thyroid tissue adjacent to the tumor (Figure 1). These were clearly distinguished from adjacent blood vessels, which did not stain for LYVE-1, and which were regular in shape and surrounded by smooth muscle and contained red blood cells (Figure 1B). In contrast to normal tissue, most regions of well-differentiated papillary thyroid carcinoma tumors appeared devoid of LYVE-1–positive intratumoral vessels, but further examination showed discrete hot spots of vessels within some tumors. These intratumoral vessels often had a distinctive reticular architecture with numerous tiny or ill-defined lumina (Figure 2A-C) that differed markedly from the larger and more conventional architecture of vessels found at the tu-

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**Figure 1.** A, Large, LYVE-1–positive, irregularly shaped, thin-walled lymphatics in normal thyroid tissue (hematoxylin-eosin, anti–LYVE-1 antibody, original magnification \(\times 400\)). B, Adjacent blood vessel, regular in shape, surrounded by smooth muscle, not staining for LYVE-1 (hematoxylin-eosin, anti–LYVE-1 antibody, original magnification \(\times 250\)).
mor margin (Figure 2D) or within normal tissue areas (Figure 1). Ninety-seven tumor sections were suitable for LVD evaluation. The other 12 sections demonstrated poor staining of normal lymphatics in thyroid tissue surrounding the tumor or excessive background staining, making identification of tumor lymphatics difficult.

The median age of the study patients was 44 years, ranging from 19 to 78 years, with 20 men and 77 women. The median tumor size was 2.0 cm, and the range was 0.9 to 6.5 cm. Sixty-six patients had stage N0 at presentation and 31 had metastatic spread to the regional lymph nodes. The clinical and pathological data are summarized in the Table.

The median LVD was 0, with a range of 0 to 4.33. This reflects the high number of tissue sections, 55, with no lymphatics identified anywhere in the tumor section. The mean LVD was 0.9. There was a highly significant association between the presence of intratumoral lymphatics in patients with well-differentiated thyroid carcinoma and the presence of neck node metastases at presentation ($P < .001$, Pearson $\chi^2$). There was also a significant association between the presence of intratumoral lymphatics and male sex ($P = .03$, Pearson $\chi^2$) and presence of multifocal disease ($P = .05$, Pearson $\chi^2$). There was no association between the presence of intratumoral lymphatics and the patient’s age ($P = .36$, Pearson $\chi^2$) or tumor size greater than 2 cm ($P = .48$, Pearson $\chi^2$).

When all the variables were entered into the logistic regression model, the presence of intratumoral lymphatics remained significantly associated with the presence of nodal metastases at presentation ($P = .003$; relative risk, 4.79; range, 1.72-13.33). All other variables were

### Table: Association Between Intratumoral Lymphatics and Clinicopathological Variables

<table>
<thead>
<tr>
<th>Sex</th>
<th>Absent, LVD = 0</th>
<th>Present, LVD &gt; 0</th>
<th>Pearson $\chi^2$, P</th>
</tr>
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<tr>
<td>Male</td>
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<td>7</td>
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</tr>
<tr>
<td>Female</td>
<td>77</td>
<td>48</td>
<td>29</td>
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<td>Age, y</td>
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<td></td>
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<tr>
<td>&lt;45</td>
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<td>30</td>
<td>19</td>
</tr>
<tr>
<td>≥45</td>
<td>48</td>
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<td>23</td>
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<tr>
<td>Tumor size, cm</td>
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</tr>
<tr>
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</tr>
<tr>
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<td>Multifocal tumor</td>
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Abbreviation: LVD, lymphatic vessel density.
The strong association between the presence of intratumoral lymphatics and regional lymph node metastases in this study of patients with papillary thyroid carcinoma suggests that proliferation and invasion of new lymphatics into the tumor may play a role in its spread via the lymphatics. The observation of small, irregular lymphatics within the tumor mass suggests that these are newly formed, rather than being preexisting lymphatics that have been surround and entrapped by the advancing tumor mass. The morphologic appearance of these intratumoral vessels resembles that of immature tumor blood vessels observed in other studies.21

In experimental models of lymphangiogenesis, it has been established that members of the VEGF family, specifically VEGF-C and VEGF-D, and their principal receptor VEGFR-3, play a key role in the formation of new lymphatics.22 With the use of LYVE-1 as a lymphatic endothelial cell marker, a direct role for VEGF-C and VEGF-D in promoting both intratumoral lymphangiogenesis and lymphatic metastasis has been demonstrated in animal models.9,10,11 These findings indicate that access to the lymphatic network and growth factor–induced changes in permeability may play a role in lymphatic invasion and metastasis.

With the use of LYVE-1 as the marker, a high LVD has also been associated with the presence of neck node metastases in head and neck squamous cell carcinoma,8 much as was demonstrated in this study with papillary thyroid carcinoma. It is difficult from a study like this to demonstrate that intratumoral lymphangiogenesis plays a direct role in the spread of tumor to regional lymph nodes, as archived pathological specimens are simply a snapshot of time and a very limited sample of the tumor is examined at any one time. However, the strong association observed between the presence of intratumoral lymphatics in this and other studies suggests that the presence of morphologically abnormal lymphatics adjacent to tumor cells may facilitate the tumor’s spread to regional lymph nodes.

Inhibition of lymphangiogenesis is a new opportunity for antimetastatic therapy.23 Neutralizing antibodies against VEGFR-3, VEGF-C, or VEGF-D or low-molecular-weight inhibitors of VEGFR-3 all show the ability to prevent the proliferation and invasion of lymphatics into and around tumors and prevent lymphatic metastases in animal models.9,11,24 Antilymphangiogenesis therapy may in the future form part of the wider-ranging attack on many aspects of tumor physiology and molecular biology, including growth, immortality, and angiogenesis. This study indicates an important way in which primary thyroid carcinomas may spread to regional lymph nodes and in the future may provide an additional target for therapy, particularly in the management of residual disease after definitive treatment.

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REFERENCES