2011 Young Surgeon's Award Winner: High Endothelial Venules: A Novel Prognostic Marker in Cancer Metastasis and the Missing Link?

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Abstract

**Introduction:** The extent of lymph nodes (LNs) metastasis is a major determinant for the staging and the most reliable adverse prognostic factor. Primary tumours can induce lymphatics and vasculature reorganisations within sentinel LN before the arrival of cancer cells and these key blood vessels are identified as high endothelial venules (HEV). The alterations of HEV in the presence of cancer, coupled with the increased proliferation rate of the endothelial cells, results in a functional shift of HEV from immune response mediator to blood flow carrier. We aim to evaluate tumour-induced vascularisation in regional LN of cancer patients by studying the morphological and functional alterations of HEV and its correlation to clinico-pathological features. **Materials and Methods:** This multi-centre study with a prospective database identified 65 consecutive patients with tongue squamous cell carcinoma (SCC) who underwent primary surgical treatment from 2001 to 2005. Immunohistochemical staining for HEV and image analysis were performed and analysed with correlation to the patients' clinico-pathological features. **Results:** The total number of HEV is significantly associated to disease-free interval when controlling for the group (P = 0.022) as well as combining both groups as one cohort (P = 0.023). There is also a similar association comparing the HEV parameters to overall survival. **Conclusion:** Our results suggest that HEV possibly plays a key role in the pathogenesis of lymphatic and subsequent distant metastases and may provide the missing link in cancer metastasis. Confirmation of this hypothesis would offer a novel therapeutic approach to preventing metastasis by blocking the remodeling processes of HEV in LN.

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Key words: High endothelial venules, Cancer metastasis, Angioegenesis

**Introduction**

Oral and pharyngeal cancer is ranked as the eighth most common cancer diagnosed in men in the United States, and tongue is the most prevalent site. Despite advances in surgery and radiation therapy, the 5-year survival rate has not improved significantly over the past several decades and remains at 50-55%. Sentinel lymph node (SLN) metastasis is the initial step in cancer metastasis and is the doorway to the regional node basin. The SLN undergoes changes induced by the primary tumour and there are vasculature and lymph channel reorganisations even before the arrival of cancer cells. The key blood vessels in lymph nodes (LN) that are remodeled are identified as high endothelial venules (HEV). Tumour-reactive lymphadenopathy in SLN has been observed for decades, but alterations of the lymphatic channels and vasculature in these nodes before the arrival of metastatic tumour cells remain unexplored and not well characterised.

The presence of metastatic cells in regional LN along with extracapsular spread of the cervical LN are the most important prognostic factors in patients with squamous cell carcinoma (SCC) of the tongue. The lymphatic metastatic cascade is a series of complex interrelated steps and processes. The relationship between angiogenesis and lymphangiogenesis is extensively studied in cancer...
This is pertinent in the biology of LN metastasis as the two systems lie side by side. There is direct evidence showing members of the vascular endothelial growth factor (VEGF) family are not only important regulators of lymph vessel growth but also enhance lymphatic metastasis.\textsuperscript{11-14} This is significant because there is evidence that tumour can activate both LN lymphangiogenesis and angiogenesis before they metastasise, this translates into efforts in this neglected field of anti-cancer research targeting pathways of tumour lymphangiogenesis and angiogenesis. There are encouraging results from therapeutic targeting of the VEGF receptors and their pathways in cancer treatment.\textsuperscript{11,15-20}

HEV are specialised post-capillary venules found in the para-cortical areas of LNs. They are distinct morphologically and functionally from ordinary venules and they are well characterised in the field of immunology.\textsuperscript{21,22} These studies suggest that HEV has a central role in lymphocyte trafficking to LN. They allow the entry of native L-selectin high cells into the LN parenchyma and this is mediated by chemokines around HEV such as the peripheral node addressins (PNAd). There is a synchrony between HEV and lymphatic vessels as revealed by immunisation studies. LNs undergo remodeling functionally with changes in kinetics of lymph flow, cell content flow, blood flow and HEV gene expression.\textsuperscript{21} Recently, with animal models, it was shown that before the arrival of metastasis in the SLN, there are reorganisations of vasculature and lymphatic channels resulting in the SLN to become a functional blood vessel enriched organ. These prominent blood vessels are remodeled HEV.\textsuperscript{5} The extent of lymph sinus dilation correlated with the primary tumour weight and this is consistent with findings from other studies that, in contrast to angiogenesis, where flow occurs only after development of vessels, lymphangiogenesis can be induced by interstitial fluid channeling. The analogous role of HEV in immune function and cancer metastasis ends here.\textsuperscript{6} In inflammatory conditions, HEV’s chief role in the traffic control of lymphocytes is evident from the presence of lymphocytes in the dilated lymphatic sinuses; whereas in tumour-reactive lymphadenopathy, there are few cells, suggesting a different process in cancer.\textsuperscript{6} This may be due to a change of HEV’s role in the presence of malignancy. There are also studies in murine models to suggest that the movement of tumour cells to LN resembles the normal migration of dendritic cells during immune stimulation, resulting in the term ‘tumour cell trafficking’.\textsuperscript{23} This observation coupled with the knowledge of the intimate relationship between HEV and lymphatic vessels in LN leads us to the logical hypothesis that HEV might provide the shortcut or a bypass route connecting the vascular and lymphatic system at the level of the SLN. This is in contrast to the Halstedian philosophy that an enbloc resection of the primary cancer and its regional lymphatic basin will achieve cure as its assumption is that tumour cells follow a stepwise pathway, from the primary tumour to the regional LNs, to the next echelon and then to the systematic circulation through distal lymphaticovenous connections such as the thoracic duct. We know that this orderly fashion is not all true, as from clinical follow-up studies, for example, about 20\% of women with node-negative breast cancer go on to develop distant metastases.\textsuperscript{24,25} The HEV providing the vehicle of this shortcut route for the cancer cells might account for this subset of these patients and explain this observed phenomenon.

In addition to this hypothetical role of providing the physical shortcut route in the LNs, in the presence of a tumour, the presence of intra-luminal red blood cells and the significant increase in lumen suggests that HEV functions like a blood vessel in anticipation to supply the needs for an accelerating growth of a soon-to-arrive tumour deposit. This may explain why the tumour in nodes are frequently larger and faster growing than the primary cancer itself commonly seen in the cases of nasopharyngeal carcinomas.\textsuperscript{26} This phenomenon is also consistent with the better chemotherapy and radiotherapy responses in metastatic lesions of the breast and head and neck cancers, in part due to the lower proportion of hypoxic cancer cells and the increased delivery of the chemotherapeutic agents as a result of a better blood supply.\textsuperscript{6}

We aim to evaluate the tumour-induced vascularisation in regional LNs of the patients with tongue cancer, confirm

\begin{figure}
\centering
\includegraphics[width=\textwidth]{Fig_1.png}
\caption{Metamorphosis of high endothelial venules (HEV) in a tumour microenvironment. This process begins with the HEV increasing in absolute numbers, then each of them becoming more dilated and lastly, every one of them will become a function vessel.}
\label{fig:HEV}
\end{figure}
its morphological and functional alterations and correlate these findings with clinical outcome. This transformation of HEV, shifting from immune modulator to blood-flow carrier, in the presence of cancer is a spectrum (Fig. 1) and if these morphological features of altered HEV correlate well with clinical outcome, this will establish its pivotal role in the pathogenesis of metastasis and support our hypothesis that HEV may be the missing link in the cascade of lymphatic metastasis. This will also serve HEV as a novel prognostic marker and a potential candidate for therapeutic targeting. In the ‘seed and soil’ theory of cancer metastasis, HEV will be the fertiliser in the “soil” creating a rich environment for the propagation and growth of the “seed” when it arrives.

Methods

Patients

This is a multi-centre study based on 175 consecutive patients with SCC of the head and neck who have undergone primary surgical treatment at X Hospital and the X Centre from January 2001 to December 2005. A review of these patients’ pathological and clinical data including follow-up information was performed from a prospective surgical database. Our inclusion criteria includes all surgically treated patients with histologically proven SCC tongue with any form of neck dissection. Exclusion criteria includes non-SCC tongue cancers, any previously radiated or treated necks, a second primary cancer, follow-up period of less than 2 years and patients without surgery and a neck dissection as part of their primary treatment. There are 76 patients stratified according to the presence or absence of pathologically proven LN metastases. Out of these, 65 patients were included and 11 patients were excluded because of either the unavailability of their tissue paraffin blocks and/or the lack of sufficient clinical and follow-up data. There were 35 patients in the group with primary SCC tongue without pathologically proven lymph node metastases in their neck dissection specimen. They were designated as ‘cases’. Thirty patients in the other group with primary SCC tongue and pathologically proven lymph node metastases in the neck dissection specimens were designated as ‘controls’. All patients had radical excision of the primary tongue lesion and a form of neck dissection (either a unilateral or bilateral, supraomohyoid neck dissection or a modified radical neck dissection). Tumours were classified according to the AJCC TNM Staging Classification.3

Results

To study the association of HEV and patient’s outcome, we first analysed the OS and DFI of the 2 groups prior to individually analysing the various HEV parameters (A, B, C, B/A, C/B, C/A, as defined in the Methods section) against OS and DFI. These analyses were repeated without controlling...
for the group factor (ie. taking the whole sample population as a single cohort). Finally, we investigated the various HEV parameters in each of the groups and determined if there was a difference in the HEV parameters, if there was presence or absence of a metastasis in the LN.

Overall Survival Analysis

We compared the OS between cases and controls and analysed the relative risk. The risk of case group is estimated to be 0.229 times of the risk of control group based on the study sample (a 95%, CI is 1.007~1.102). In another words, the patients with presence of metastases in their regional cervical LNs have a 4.367 higher chance of dying as compared to patients without metastasis in their regional LNs. This is significant in terms of overall survival time between controls and cases ($P = 0.046$) (Fig. 2). There was no significance found in the total no. of HEV (A); no. of dilated HEV (B); no. of dilated HEV with RBCs inside

**Table 1. Summary of Results**

<table>
<thead>
<tr>
<th>HEV parameters</th>
<th>Clinical data</th>
<th>Relative risk</th>
<th>$P$ value</th>
<th>95% confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total no. of HEV (A)</td>
<td>Overall Survival</td>
<td>1.024</td>
<td>0.471</td>
<td>0.961 – 1.091</td>
</tr>
<tr>
<td></td>
<td>Disease Free Interval</td>
<td>1.051</td>
<td>0.022</td>
<td>1.007 – 1.097</td>
</tr>
<tr>
<td>Total no. of HEV (A) and Disease Free Interval (as a Cohort)</td>
<td>Disease Free Interval</td>
<td>1.051</td>
<td>0.023</td>
<td>1.007 – 1.096</td>
</tr>
<tr>
<td>Dilated HEV (B)</td>
<td>Overall Survival</td>
<td>1.071</td>
<td>0.476</td>
<td>0.886 – 1.295</td>
</tr>
<tr>
<td></td>
<td>Disease Free Interval</td>
<td>1.034</td>
<td>0.594</td>
<td>0.915 – 1.169</td>
</tr>
<tr>
<td>HEV with RBCs within its lumen (C)</td>
<td>Overall Survival</td>
<td>1.116</td>
<td>0.345</td>
<td>0.889 – 1.401</td>
</tr>
<tr>
<td></td>
<td>Disease Free Interval</td>
<td>1.044</td>
<td>0.584</td>
<td>0.896 – 1.216</td>
</tr>
<tr>
<td>Ratio of dilated HEV to the total no. of HEV (B/A)</td>
<td>Overall Survival</td>
<td>1.078</td>
<td>0.982</td>
<td>0.002 – 638.23</td>
</tr>
<tr>
<td></td>
<td>Disease Free Interval</td>
<td>10.10</td>
<td>0.450</td>
<td>0.001 – 39.89</td>
</tr>
<tr>
<td>Ratio of dilated HEV with RBCs to total no. HEV (C/A)</td>
<td>Overall Survival</td>
<td>3.624</td>
<td>0.737</td>
<td>0.002 – 6634.42</td>
</tr>
<tr>
<td></td>
<td>Disease Free Interval</td>
<td>4.67</td>
<td>0.643</td>
<td>0.001 – 145.83</td>
</tr>
<tr>
<td>Ratio of dilated HEV with RBCs within its lumen to total no. of dilated HEV (C/B)</td>
<td>Overall Survival</td>
<td>17.884</td>
<td>0.171</td>
<td>0.287 – 1114.67</td>
</tr>
<tr>
<td></td>
<td>Disease Free Interval</td>
<td>5.458</td>
<td>0.208</td>
<td>0.389 – 76.616</td>
</tr>
</tbody>
</table>

HEV: high endothelial venules; RBCs: red blood cells
its lumen (C) or any of the HEV ratios (B/A, C/B, C/A) with respect to the OS (Table 1).

**Disease Free Interval Analysis**

Similarly, we compared the DFI between controls and cases. The risk of case group is estimated to be 0.75 times of the risk of control group based on the sample (a 95% CI is 0.342–1.644). This implies that the DFI tend to be longer in patients without LN metastases. However, this did not achieve statistical significance ($P = 0.472$) (Fig. 2).

There is a statistically significant association of number of HEV (A) to the DFI. This is statistically significant when controlling for the group (control vs. case) ($P = 0.022$). There is also statistical significance when we combined both group as 1 cohort (control + case) ($P = 0.023$). However, there was no significance found in the other HEV parameters, namely no. of dilated HEV (B); no. of dilated HEV with RBCs inside its lumen (C) or any of the HEV ratios (B/A, C/B, C/A) with respect to the DFI (Table 1).

We also investigated the various HEV parameters in each of the groups and determined if there was a difference in the HEV parameters’ values, if there was presence or absence of a metastasis in the LN. The average ratio of dilated HEV with RBCs within its lumen to total no. of dilated HEV (C/B) in control groups is statistically significantly higher than that in case group ($P = 0.0318$) (Table 2).

**Table 2. Comparing HEV Parameters in the 2 Groups**

<table>
<thead>
<tr>
<th>HEV parameters</th>
<th>Control (n = 30)</th>
<th>Case (n = 35)</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total number of HEV (A)</td>
<td>40.085 / 1.83</td>
<td>41.305 / 1.59</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Mean/s.e.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ratio of dilated HEV to the total number of HEV (B/A)</td>
<td>0.149 / 0.0086</td>
<td>0.166 / 0.0186</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Ratio of dilated HEV with RBCs within its lumen with respect to total no. of dilated HEV (C/A)</td>
<td>0.098 / 0.0078</td>
<td>0.101 / 0.0135</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Ratio of dilated HEV with RBCs within its lumen to total no. of dilated HEV (C/B)</td>
<td>0.646 / 0.0301</td>
<td>0.582 / 0.0275</td>
<td>0.0318</td>
</tr>
</tbody>
</table>

**Discussion**

SCC of the tongue is one of the most prevalent tumours of the head and neck region and is one of the cancers with fast rising rate today affecting especially the young.1,2,29,30 The prognosis is worse compared to other equivalent carcinomas of the head and neck region as it has a high propensity for LN metastases, which serves tongue cancer as the ideal cancer to investigate our hypothesis on HEV’s role. There are several clinico-pathological factors proposed as prognostic in oral SCC, such as stage of primary tumour, site, extension, thickness, grade of histological differentiation and perineural invasion. Of all these factors, the presence of cervical LN metastasis and extracapsular spread are the most important adverse prognostic factors.

It has been proposed that SLN biopsy may play the next stage in the evolution in the neck management and treatment of tongue SCC.31 Understanding this concept coupled with advances in anti-angiogenesis therapy translates the next logical exploration to be the study of the pathogenesis of LN metastasis. It was shown recently that the lumens of the lymphatic sinuses and blood vessels were dilated in SLN before metastasis.3 We have demonstrated that LNs are transformed by the primary tongue cancer to become a functional blood vessel-enriched organ before and independent of metastasis, with the changes in HEV morphology consistent with it being the main blood flow carrier in the LN (Fig. 1). This process of vascularisation in the LN (ie. the HEV morphological alterations) appears similar and consistent in human tissues and previous animal models.5 This study presents functional and structural data that the primary tumour is manipulating LNs’ microenvironment and biology to improve the acceptance and proliferation of subsequent tumour deposits leading to established metastases.

By expressing homing receptors on their surface, which blood lymphocytes can recognise as they pass in circulation, HEV provide a unique location where naive lymphocytes can enter the LN.32 In a previous study, the role of HEV was transformed from a lymphocyte recruiter to become the main blood flow carrier in the SLN prior to metastasis.3 This phenomenon is verified in this study, not only could the HEV morphology change dramatically to carry more blood flow, but the proliferation rate of HEV endothelial cells was also increased before metastasis. The transformation of HEV as a lymphocytic carrier in LNs to a blood-flow carrier is evident from the large quantity of red blood cells visible in the HEV. This phenomenon is more pronounced in the patients with established LN metastasis. We demonstrated this in our analysis that patients with LN metastases have more dilated HEV with RBCs in their LN as compared to patients without LN metastasis. The average ratio of dilated HEV with RBCs within its lumen to total no. of dilated HEV...
In addition, with the benefit of patients’ clinical information, we have demonstrated statistically that even with an increase of 1 HEV in a high-power-field (HPF), regardless of the group, the risk is 1.024 times worse in terms of OS (Table 3). This is also consistent with clinical knowledge and natural history of the disease. We demonstrated that patients without LN metastases in their regional LN have a significantly better prognosis (Fig. 2).

Assuming that our hypothesis is true, HEV transformation from a normal immunological mediator to a tumour metastasis mediator is reflected by morphological changes from a normal appearing HEV to a dilated HEV to lastly a dilated HEV containing RBCs. We believe that this metamorphosis is a spectrum and this process begins with the HEV increasing in absolute numbers, then each of them becoming more dilated and finally every one of them will become a function blood vessel (Fig. 1). We analysed our data, looking at the 3 different stages of HEV transformation and the ratio comparing each parameter in a systematic way to demonstrate this spectrum (Fig. 1).

In our study, we found that this spectrum of metamorphosis is consistent with the patients’ OS regardless of their LN status. We illustrated that a patient’s OS risk is 1.024 times worse than an equivalent patient if there is 1 more HEV in a HPF of his regional LN, the risk is increased to 1.071 if the HEV is a dilated HEV. The risk is even higher at 1.116 times if the HEV is a dilated HEV containing RBCs (Table 1). This trend is also reflected if we considered all the patients as one cohort.

In the treatment of tongue carcinomas and head and neck cancers, DFI is important as it signifies the failure of loco-regional control. DFI is also analysed with respect to the 3 different HEV morphological phenotypes (A, B, C) (Fig. 1). We found statistical significance in the relationship between the total number of HEV (A) and DFI when we controlled for the group ($P = 0.022$). This significance is preserved when we analysed the patients as one cohort ($P = 0.023$). This signifies the quantity of HEV is inversely related to DFI regardless of LN metastasis status (Table 1).

There is a general trend observed: the more advanced the disease is, the higher the ratio/percentage of abnormality of HEV. This can be seen when we analysed OS and the 2 ratios. The OS relative risk worsens by 1.078 if the ratio B/A increased by 1, the OS relative risk worsens by 3.624 times if the ratio C/B increased by 1. Finally, if we consider the most abnormal HEV phenotype, a patient’s OS relative risk worsens by 17.884 times if this ratio (C/A) increases by a factor of 1. This observation approaches marginal significance ($P = 0.171$) (Fig. 3).

In this study, we have shown the relationship of HEV in the control (pN+) group is statistically significantly higher than that in case group ($P = 0.0318$).

In addition, with the benefit of patients’ clinical information, we have demonstrated statistically that even with an increase of 1 HEV in a high-power-field (HPF), regardless of the group, the risk is 1.024 times worse in terms of OS (Table 3). This is also consistent with clinical knowledge and natural history of the disease. We demonstrated that patients without LN metastases in their regional LN have a significantly better prognosis (Fig. 2).

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In our study, we found that this spectrum of metamorphosis is consistent with the patients’ OS regardless of their LN status. We illustrated that a patient’s OS risk is 1.024 times worse than an equivalent patient if there is 1 more HEV in a HPF of his regional LN, the risk is increased to 1.071 if the HEV is a dilated HEV. The risk is even higher at 1.116 times if the HEV is a dilated HEV containing RBCs (Table 1). This trend is also reflected if we considered all the patients as one cohort.

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Table 3. Illustration of the Exponential Effect of HEV(A) on the Overall Survival

<table>
<thead>
<tr>
<th>No. of HEV (A) in either a CONTROL or CASE group</th>
<th>Control (0) or Case (1)</th>
<th>No. of HEV(A)</th>
<th>Relative risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of HEV(A) in a CONTROL group patient = 0</td>
<td>Control (0) or Case (1)</td>
<td>0</td>
<td>1.00</td>
</tr>
<tr>
<td>No. of HEV(A) in a CONTROL group patient = 1</td>
<td>Control (0) or Case (1)</td>
<td>0</td>
<td>1.024</td>
</tr>
<tr>
<td>No. of HEV(A) in a CONTROL group patient = 2</td>
<td>Control (0) or Case (1)</td>
<td>0</td>
<td>1.049</td>
</tr>
<tr>
<td>No. of HEV(A) in a CONTROL group patient = 100</td>
<td>Control (0) or Case (1)</td>
<td>0</td>
<td>10.715</td>
</tr>
<tr>
<td>No. of HEV(A) in a CASE group patient = 0</td>
<td>Control (0) or Case (1)</td>
<td>1</td>
<td>0.428</td>
</tr>
<tr>
<td>No. of HEV(A) in a CASE group patient = 1</td>
<td>Control (0) or Case (1)</td>
<td>1</td>
<td>0.428 x 1.024</td>
</tr>
<tr>
<td>No. of HEV(A) in a CASE group patient = 2</td>
<td>Control (0) or Case (1)</td>
<td>1</td>
<td>0.428 x (1.024)$^2$</td>
</tr>
</tbody>
</table>

To observe the association of total no. of HEV (A) on overall survival while controlling for the group (control vs. case), we looked at the relative risk in the above output. While controlling for the group, the risk is estimated to be 1.024 times when the HEV value increases by 1 based on the sample (a 95%, C.I. is 0.961 ~ 1.091). However, the association on HEV and overall survival is not significant while controlling for group ($P = 0.955$).

(C/B) in the control (pN+) group is statistically significantly higher than that in case group ($P = 0.0318$).

Fig. 3. Overall survival relative risk with respect to the different HEV ratios: A: no. of all HEV; B: no. of dilated HEV (defined as lumen size more than 80 square micron); C: no. of dilated HEV with red blood cells (RBCs) inside its lumen; B/A: Ratio of dilated HEV to the total number of HEV; C/B : Ratio of dilated HEV with RBCs within its lumen to total no. of dilated HEV; C/A : Ratio of dilated HEV with RBCs within its lumen with respect to total no. of HEV.
and their transformation in a cancerous environment. In previous studies the HEV morphology did not alter in endotoxin-induced lymphadenopathy, implying a selective reaction of HEV in the cancerous condition. The blood vessel endothelium has tremendous potential to adapt its environment, as a consequence, HEV was remodeled from a thick walled, endothelial vessel with a small lumen to a thin walled, large-lumen vessel (Fig. 1), shifting its function from recruitment of lymphocytes to become a blood vessel. The enlarged, remodeled HEV could integrate into the metastatic tumour vasculature with further differentiation, characterised by the gradual loss of their specific marker MECA-79 from the tumour margin to the central part of the metastatic tumour nest. Based on our findings, the metastatic tumour vasculature in LN consists of many large blood vessels derived from normal HEV, suggesting that the efficiency of nutrition and oxygen supplies could be better for the metastatic tumour cells in the involved LN. The enrichment of the blood supply in the LN before and after metastasis may favour the growth of newly arising metastatic cancer cells. Consequently as in clinical situations seen in the follow-up of cancer patients, the involved regional LNs may manifest while the primary tumours remain clinically occult for years.

Conclusion

This study demonstrates morphologic and functional alterations of the HEV to become the main blood flow carrier in the LN. The analysis reveals the relationship of HEV and their metatmorphosis in pre-metastatic and metastatic environment in regional LNs of tongue cancer patients in correlation with clinical outcomes. Our findings coupled with studies elucidating the basis of lymphangiogenesis and angiogenesis within sentinel LNs support our hypothesis that HEV play a pivotal role and may be the elusive junction providing the lymph flow shortcut routes into the systemic circulation in sentinel LNs. The confirmation of this shortcut and the exploration of the related molecular mechanism of establishing the shortcut will broaden our knowledge about lymph circulation in cancerous conditions and may provide novel therapeutic targets.

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