

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

Ser Yee Lee,^{1,2,3} MBBS, MMed (General Surgery), MSc, FRCS (Ed), Chao Nan Qian,^{4,5} MD, PhD, Aik Seng Ooi,⁶ PhD, Peiyi Chen,⁷ MSc, Veronique KM Tan,¹ MBBS, MMed (General Surgery), MRCS, Claramae S Chia,¹ MBBS, MRCS, Jacqueline SG Hwang,⁸ MBBS, FRCPA, Bin Tean Teh,^{2,9} BMedSc, MD, PhD, Khee Chee Soo,^{1,3} MBBS, FRCS(Ed), MD

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.

Ann Acad Med Singapore 2012;41:21-28

Key words: High endothelial venules, Cancer metastasis, Angioogenesis

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.¹ 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%.^{2,3}

Sentinel lymph node (SLN) metastasis is the initial step in cancer metastasis and is the doorway to the regional node basin.⁴ 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).^{5,6} 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.⁷⁻¹⁰ The lymphatic metastatic cascade is a series of complex interrelated steps and processes. The relationship between angiogenesis and lymphangiogenesis is extensively studied in cancer

¹Department of General Surgery, Singapore General Hospital, Singapore

²National Cancer Centre Singapore-Van Andel Research Institute, Translational Cancer Research Laboratory, National Cancer Centre, Singapore

³Department of Surgical Oncology, National Cancer Centre, Singapore

⁴Laboratory of Cancer and Developmental Cell Biology, Van Andel Research Institute, Grand Rapids, Michigan, USA

⁵Department of Nasopharyngeal Carcinoma, Sun Yat-sen University Cancer Center, Guangzhou, Guangdong, PRC

⁶Center for Cancer Genomics and Computational Biology, Van Andel Research Institute, Grand Rapids, Michigan, USA

⁷Department of Statistics & Applied Probability, Faculty of Science, National University of Singapore

⁸Department of Pathology, Singapore General Hospital, Singapore

⁹Laboratory of Cancer Genetics, Van Andel Research Institute, Grand Rapids, Michigan, USA

Address for Correspondence: Dr Ser Yee Lee, Department of Surgical Oncology, National Cancer Centre, 11 Hospital Drive, Singapore 169610.

Email: seryee@yahoo.com

research. 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.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.^{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.^{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.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.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.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.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'.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.^{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.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.⁶

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

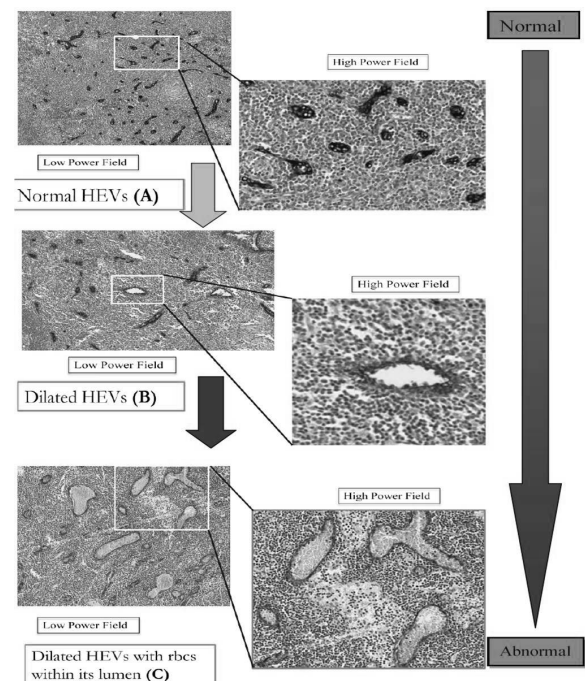


Fig. 1. Metamorphosis of high endothelial venules (HEV) in a tumour microenvironment. This process begins with the HEV increasing in absolute numbers, then each of them becoming more dilated and lastly, every one of them will become a function vessel.

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 a bilateral, supraomohyoid neck dissection or a modified radical neck dissection). Tumours were classified according to the AJCC TNM Staging Classification.²⁷ Intra-operative frozen sections were performed for margins of the tongue lesion and further resection of the tongue was performed in event that the surgical margins were involved to achieve R₀ resection. Upon discharge from the hospital, they were followed up at regular intervals of monthly, 3-monthly, 6-monthly and yearly at a progressive rate.

Immunohistochemistry

We conducted a histological review and analysis of the primary SCC tongue lesion and their respective LN tissues in the neck dissection specimen. A blinded independent head and neck pathologist (X) reviewed the histological specimens. Optimisation of the immunohistochemical staining protocol and staining of the HEV using the MECA-79 antibody, which is specific for HEV, was performed⁵.

Computer Assisted Image Analysis

We analysed digital snapshots of the LN (ScanScope, Aperio T3; ScanScope Console v.9.0. Aperio Technologies, Vista, CA, USA). The total number of HEV, abnormal HEV and HEV with presence of red blood cells were counted with the aid of imaging software (Image J programme with cell counter plug-in)²⁸.

Definitions of the HEV’s Parameters and Ratios

This is represented by the following alphabets A, B and C for the results and discussion sections (Fig. 1):

- No. of all HEV: A
- No. of dilated HEV (defined as lumen size more than 80 square micron): B
- No. of dilated HEV with red blood cells (RBCs) inside its lumen: C

In addition to these 3 parameters, we also analysed the ratios of these abnormal HEV (B and C) with respect to the total number of HEV (A) (Fig. 1). This is defined below as:

- Ratio of dilated HEV to the total number of HEV: B/A
- Ratio of dilated HEV with RBCs within its lumen to total no. of dilated HEV: C/B
- Ratio of dilated HEV with RBCs within its lumen with respect to total no. HEV: C/A

Statistical Analysis

We analysed the 3 parameters (A, B, C) and the 3 ratios (B/A, C/B, C/A) with respect to several clinical parameters, namely disease-free interval (DFI) and overall survival (OS) in the 2 groups (Cases vs. Controls) and as a whole cohort as well. We used Cox’s Proportional Hazard Model for analysis. A non-parametric test, Wilcoxon-rank-sum test, was performed to test the difference on HEV and its parameters as well as 3 ratios between control and case.

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

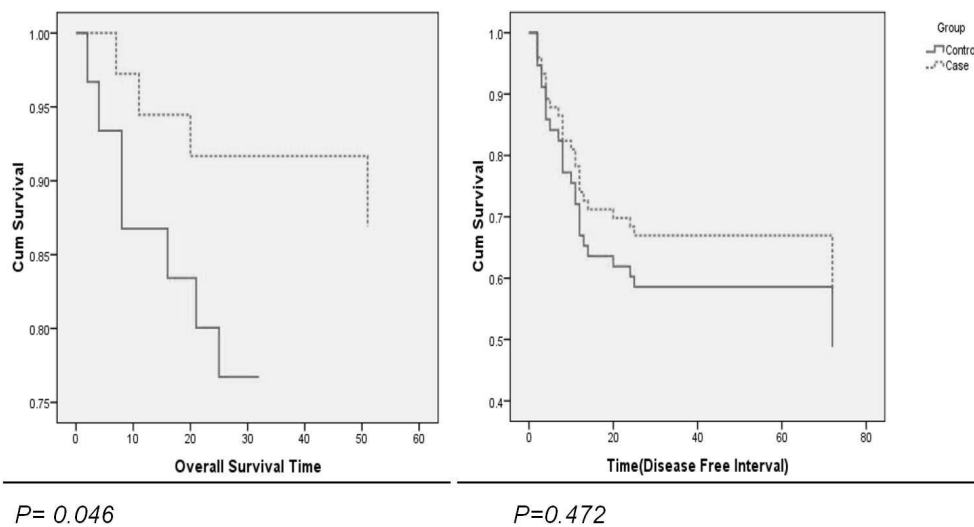


Fig. 2. Kaplan Meier Overall Survival curves and Disease Free Interval for the 2 groups (Cases vs. Controls). Cases: patients with primary SCC tongue without pathologically proven lymph node metastases in their neck dissection specimen; Controls: patients with primary SCC tongue with pathologically proven lymph node metastases in their neck dissection specimen.

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

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

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

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

HEV: high endothelial venules; s.e: standard error; Cases: patients with primary SCC tongue without pathologically proven lymph node metastases in their neck dissection specimen; Controls: patients with primary SCC tongue with pathologically proven lymph node metastases in their neck dissection specimen; RBCs: red blood cells

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.⁵ 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 models⁵. 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.⁵ 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

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

No. of HEV (A) in either a CONTROL or CASE group	Control (0) or Case (1)	No. of HEV(A)	Relative risk
No. of HEV(A) in a CONTROL group patient = 0	0	1	1.00
No. of HEV(A) in a CONTROL group patient = 1	0	1	1.024
No. of HEV(A) in a CONTROL group patient = 2	0	2	$(1.024)^2 = 1.049$
No. of HEV(A) in a CONTROL group patient = 100	0	100	$(1.024)^{100} = 10.715$
No. of HEV(A) in a CASE group patient = 0	1	0	0.428
No. of HEV(A) in a CASE group patient = 1	1	1	0.428×1.024
No. of HEV(A) in a CASE group patient = 2	1	2	$0.428 \times (1.024)^2$

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 ($P = 0.0318$).

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

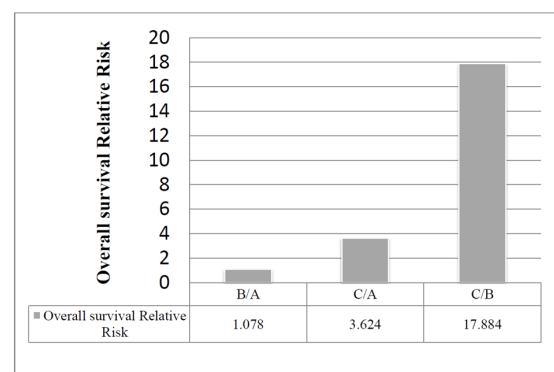


Fig. 3. Overall survival relative risk with respect to the different HEV ratios A: no. of all HEV; B: no. of dilated HEV (defined as lumen size more than 80 square micron); C: no. of dilated HEV with red blood cells (RBCs) inside its lumen; B/A: Ratio of dilated HEV to the total number of HEV; C/B : 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.^{6,33} 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.³⁴ 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 arriving 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.³⁵

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 metamorphosis 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.

Acknowledgements

The authors would like to thank the National Cancer Centre, Singapore-Van Adel Research Institute Translational Research Laboratory for providing the necessary laboratory equipments, and Research officers Ms Jacey Wong and Ms Bernice Wong for their technical and administrative support.

REFERENCES

1. Jemal A, Siegel R, Ward E, Hao Y, Xu J, Murray T, et al. Cancer statistics, 2008. *CA Cancer J Clin* 2008;58:71-96.
2. Silverman S Jr. Demographics and occurrence of oral and pharyngeal cancers. The outcomes, the trends, the challenge. *J Am Dent Assoc* 2001;132 Suppl:7S-11S.
3. Edwards BK, Brown ML, Wingo PA, Howe HL, Ward E, Ries L, et al. Annual report to the nation on the status of cancer, 1975-2002, featuring population-based trends in cancer treatment. *J Natl Cancer Inst* 2005;97:1407-27.
4. Cabanas RM. An approach for the treatment of penile carcinoma. *Cancer* 1977;39:456-66.
5. Qian CN, Berghuis B, Tsarfaty G, Bruch M, Kort EJ, Ditlev J, et al. Preparing the "soil": the primary tumor induces vasculature reorganization in the sentinel lymph node before the arrival of metastatic cancer cells. *Cancer Res* 2006;66:10365-76.
6. Qian CN, Resau JH, Teh BT. Prospects for vasculature reorganization in sentinel lymph nodes. *Cell Cycle* 2007;6:514-7.
7. Balch CM, Soong SJ, Gershenwald JE, Thompson JF, Reintgen DS, Cascinelli N, et al. Prognostic factors analysis of 17,600 melanoma patients: validation of the American Joint Committee on Cancer melanoma staging system. *J Clin Oncol* 2001;19:3622-34.
8. McGuire WL. Prognostic factors for recurrence and survival in human breast cancer. *Breast Cancer Res Treat* 1987;10:5-9.
9. Harrison JC, Dean PJ, el-Zeky F, Vander Zwaag R. From Dukes through Jass: pathological prognostic indicators in rectal cancer. *Hum Pathol* 1994;25:498-505.
10. Sano D, Myers JN. Metastasis of squamous cell carcinoma of the oral tongue. *Cancer Metastasis Rev* 2007;26:645-62.
11. Stacker SA, Caesar C, Baldwin ME, Thornton GE, Williams RA, Prevo R, et al. VEGF-D promotes the metastatic spread of tumor cells via the lymphatics. *Nat Med* 2001;7:186-91.
12. Mäkinen T, Jussila L, Veikkola T, Karpanen T, Kettunen M, Pulkkanen KJ, et al. Inhibition of lymphangiogenesis with resulting lymphedema in transgenic mice expressing soluble VEGF receptor-3. *Nat Med* 2001;7:199-205.
13. Skobe M, Hawighorst T, Jackson DG, Prevo R, Janes L, Velasco P, et al. Induction of tumor lymphangiogenesis by VEGF-C promotes breast cancer metastasis. *Nat Med* 2001;7:192-8.
14. Mandriota SJ, Jussila L, Jeltsch M, Compagni A, Baetens D, Prevo R, et al. Vascular endothelial growth factor-C-mediated lymphangiogenesis promotes tumour metastasis. *EMBO J* 2001;20:672-82.
15. Fukumoto S, Morifuji M, Katakura Y, Ohishi M, Nakamura S. Endostatin inhibits lymph node metastasis by a down-regulation of the vascular endothelial growth factor C expression in tumor cells. *Clin Exp Metastasis* 2005;22:31-8.
16. Roberts N, Kloos B, Cassella M, Podgrabinska S, Persaud K, Wu Y, et al. Inhibition of VEGFR-3 activation with the antagonistic antibody more potently suppresses lymph node and distant metastases than inactivation of VEGFR-2. *Cancer Res* 2006;66:2650-7.
17. Zwaans BM, Bielenberg DR. Potential therapeutic strategies for lymphatic metastasis. *Microvasc Res* 2007;74:145-58.
18. Faivre S, Demetri G, Sargent W, Raymond E. Molecular basis for sunitinib efficacy and future clinical development. *Nat Rev Drug Discov* 2007;6:734-45.
19. Escudier B, Eisen T, Stadler WM, Szczylik C, Oudard S, Siebels M, et al. Sorafenib in advanced clear-cell renal-cell carcinoma. *N Engl J Med* 2007;356:125-34.
20. Motzer RJ, Michaelson MD, Redman BG, Hudes GR, Wilding G, Figlin

- RA, et al. Activity of SU11248, a multitargeted inhibitor of vascular endothelial growth factor receptor and platelet-derived growth factor receptor, in patients with metastatic renal cell carcinoma. *J Clin Oncol* 2006;24:16-24.
21. Liao S, Ruddle NH. Synchrony of high endothelial venules and lymphatic vessels revealed by immunization. *J Immunol* 2006;177:3369-79.
 22. Baekkevold ES, Roussigné M, Yamanaka T, Johansen FE, Jahnsen FL, Amalric F, et al. Molecular characterization of NF-HEV, a nuclear factor preferentially expressed in human high endothelial venules. *Am J Pathol* 2003;163:69-79.
 23. Muller A, Homey B, Soto H, Ge N, Catron D, Buchanan ME, et al. Involvement of chemokine receptors in breast cancer metastasis. *Nature* 2001;410:50-6.
 24. Rosen PP, Saigo PE, Braun DW Jr, Weathers E, DePalo A. Predictors of recurrence in stage I (T1N0M0) breast carcinoma. *Ann Surg* 1981;193:15-25.
 25. Leong SP. Paradigm shift of staging and treatment for early breast cancer in the sentinel lymph node era. *Breast J* 2006;12:S128-33.
 26. Zhang L, Zhao C, Peng PJ, Lu LX, Huang PY, Han F, et al. Phase III study comparing standard radiotherapy with or without weekly oxaliplatin in treatment of locoregionally advanced nasopharyngeal carcinoma: preliminary results. *J Clin Oncol* 2005;23:8461-8.
 27. Greene FL, Page DL, Fleming ID, et al. *AJCC Cancer Staging Manual*. 6th ed. New York, NY: Springer-Verlag; 2002.
 28. Qian CN, Zhang CQ, Guo X, Hong MH, Cao SM, Mai WY, et al. Elevation of serum vascular endothelial growth factor in male patients with metastatic nasopharyngeal carcinoma. *Cancer* 2000;88:255-61.
 29. Macfarlane GJ, Boyle P, Scully C. Rising mortality from cancer of the tongue in young Scottish males. *Lancet* 1987;2:912.
 30. Shemen LJ, Klotz J, Schottenfeld D, Strong EW. Increase of tongue cancer in young men. *JAMA* 1984;252:1857.
 31. Pitman KT. Sentinel node localization in head and neck tumors. *Semin Nucl Med* 2005;35:253-6.
 32. Yeh JC, Hiraoka N, Petryniak B, Nakayama J, Ellies LG, Rabuka D, et al. Novel sulfated lymphocyte homing receptors and their control by a Core1 extension beta 1,3-N-acetylglucosaminyltransferase. *Cell* 2001;105:957-69.
 33. Chi JT, Chang HY, Haraldsen G, Jahnsen FL, Troyanskaya OG, Chang DS, et al. Endothelial cell diversity revealed by global expression profiling. *Proc Natl Acad Sci USA* 2003;100:10623-8.
 34. Lacorre DA, Baekkevold ES, Garrido I, Brandtzaeg P, Haraldsen G, Amalric F, et al. Plasticity of endothelial cells: rapid dedifferentiation of freshly isolated high endothelial venule endothelial cells outside the lymphoid tissue microenvironment. *Blood* 2004;103:4164-72.
 35. Nieder C, Ang KK. Cervical lymph node metastases from occult squamous cell carcinoma. *Curr Treat Options Oncol* 2002;3:33-40.