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# Significance of Lymph Node Metastasis in Cancer Dissemination of Head and Neck Cancer[1]

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## Abstract

Lymph node metastasis (LNM) in many solid cancers is a well-known prognostic factor; however, it has been debated whether regional LNM simply reflects tumor aggressiveness or is a source for further tumor dissemination. Similarly, the metastatic process in head and neck cancer (HNC) has not been fully evaluated. Thus, we aimed to investigate the relative significance of LNM in metastatic cascade of HNC using functional imaging of HNC patients and molecular imaging in *in vivo* models. First, we analyzed $^{18}$Fluorodeoxyglucose positron emission tomography (PET) parameters of 117 patients with oral cancer. The primary tumor and nodal PET parameters were measured separately, and survival analyses were conducted on the basis of clinical and PET variables to identify significant prognostic factors. In multivariate analyses, we found that only the metastatic node PET values were significant. Next, we compared the relative frequency of lung metastasis in primary ear tumors *versus* lymph node (LN) tumors, and we tested the rate of lung metastasis in another animal model, in which each animal had both primary and LN tumors that were expressing different colors. As a result, LN tumors showed higher frequencies of lung metastasis compared to orthotopic primary tumors. In color-matched comparisons, the relative contribution to lung metastasis was higher in LN tumors than in primary tumors, although both primary and LN tumors caused lung metastases. In summary, tumors growing in the LN microenvironment spread to systemic sites more commonly than primary tumors in HNC, suggesting that the adequate management of LNM can reduce further systemic metastasis.

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## Introduction

Lymph node metastasis (LNM) in many solid cancers including head and neck cancer (HNC) is a well-known and clinically accepted prognostic factor [1,2]. However, it has been debated whether LNM reflects tumor aggressiveness or invasiveness or is a foothold for further tumor dissemination [3]. The early concept of a metastatic cascade in solid cancers was the sequential progress of tumors from the primary sites to lymph nodes (LNs) and then systemically distant organs (Halstedian theory) [4,5]. In contrast, the systemic theory of cancer metastasis highlighted the view that cancer is a systemic disease, and cancer cells disperse throughout the body at the very early phase of tumor formation [6,7]. According to this theory, the status of LNM only provides prognostic information; therefore, surgical removal of metastatic nodes does not affect patient survival. However, many clinical observations of breast [8], stomach [9], endometrial [10], and esophageal cancers [11] have not fit well into these two categories, and a spectrum theory was proposed explaining that tumor cells gain more metastatic capabilities as the tumor progresses to regional LNs [3,12] (Figure S1). Halstedian theory does not accept the direct dissemination of tumor from primary tumor to systemic sites [5]; however, the spectrum theory describes systemic tumor dissemination both from primary tumors and LNM but supposes that tumor cells spread more to systemic sites from LNM, which can be a major source of systemic disease [3].

In HNC, there have been no clear data, but similarly the active control of regional as well as local diseases is recommended for better survival of HNC patients [National Comprehensive Cancer Network (NCCN) Clinical Practice Guideline in Oncology, Head and Neck Cancer, Version 2.2013]. Molecular and genetic characteristics of cancer cells can be the main determinants of metastasis in HNC [13], and the active dissemination to blood and lymphatic vessels in the primary tumor was suggested [7]. However, the relative significance of the established LNM in further cancer dissemination of HNC has never been studied fully.

Regional LNM is a major prognostic factor for HNC, because it indicates aggressive tumor biology, as well as represents a source of subsequent metastasis (as explained by the spectrum theory) [3,14]. In addition, the tumor biology and phenotype within the primary site microenvironment can differ from those of the metastatic LNs [15–17]. More importantly, tumor cells in different microenvironments have been reported to respond differently to therapy [17–19]. Thus, increasing evidence indicates that to determine optimal treatment and to better predict prognosis, evaluation of cancer patients should be refined on the basis of the primary tumor and metastatic microenvironment.

Despite this body of knowledge, the significance of LNM and LN microenvironment has not been evaluated. Thus, the aims of this study were to provide clinical and experimental evidence regarding the role of established LNM in the metastatic cascade of HNC by analyzing functional imaging in HNC patients and using molecular imaging in *in vivo* models. Understanding metastasis progression of HNC in tumor site–specific microenvironments can lead to personalized treatments and refine the design of many clinical trials enrolling patients with metastatic/recurrent HNC.

## Materials and Methods

### *Evaluation of $^{18}$F-FDG PET/CT Imaging in Oral Cancer Patients*

First, we evaluated the $^{18}$Fluorodeoxyglucose positron emission tomography (18F-FDG PET)/computed tomography (CT) imaging in HNC patients. Our study population was limited to oral cavity squamous cell carcinoma patients, because the standard treatment is initial surgery and/or post-operative adjuvant treatment, which enabled us to access pathologic information. Newly diagnosed oral cavity cancer patients were prospectively enrolled in the study from 2006 to 2012. All participants provided written informed consent before the study. The diagnosis of oral cavity squamous cell carcinoma was confirmed by surgical pathology in all subjects. Patients with other pathology types, uncontrolled diabetes, or high blood glucose level (>200 mg/dl), secondary malignancies, or who failed to receive definitive treatment for disease were excluded from the analyses ($N$ = 20). Finally, 117 patients were included in this study. All patients were subjected to curative resection of the primary tumor with neck LN dissection ($N$ = 71) or sentinel LN biopsy ($N$ = 46). The demographics and clinicopathologic characteristics of the patients are summarized in Table S1. Whole-body $^{18}$F-FDG PET and unenhanced CT images were acquired using integrated PET/CT scanners according to the standard protocols (Discovery LS or Discovery STE; GE Healthcare, Pittsburgh, PA).

### *Measurement of $^{18}$F-FDG PET Variables*

$^{18}$F-FDG PET measurements were performed using the Volume Viewer software on the GE Advantage Workstation version 4.4 (GE Healthcare). This software automatically determines the volume of interest using an iso-contour threshold method based on the standardized uptake value (SUV). The volumes of interest were placed over the target lesions within the primary sites as well as all suspicious metastatic LNs, and the software subsequently measured the maximum SUV ($SUV_{max}$), average SUV, and metabolic tumor volume (MTV). The MTV represents a volumetric measurement of tumor cells with high glycolytic activity and was defined as the tumor volume segmented with $^{18}$F-FDG uptake above a threshold SUV of 2.5. We measured the $SUV_{max}$ and MTV of the primary tumor ($pSUV_{max}$ and pMTV) and metastatic LN ($nSUV_{max}$ and nMTV) on the pretreatment scan. We also calculated total lesion glycolysis (TLG) as the product of MTV and average SUV in both the primary tumor (pTLG) and metastatic LN (nTLG). In multiple metastatic nodes, $nSUV_{max}$ reflected the highest SUV among metastatic nodes, whereas nMTV and nTLG indicated the sum of all nodes.

If the target lesion was not visualized or could not be distinguished from background, $SUV_{max}$ and MTV values were set as zero. When the target lesion was visible but the $SUV_{max}$ was less than 2.5, the MTV was set as a single voxel with a volume of 0.05 $cm^3$.

### *Statistical Analyses*

To estimate the predictive performance of $^{18}$F-FDG PET parameters, for time-to- event data, we used time-dependent receiver operating characteristic (ROC) curve [20–22]. $^{18}$F-FDG PET parameters were stratified using optimal cutoff values based on the highest Youden's index from time-dependent ROC curves at 24 months [23]. Univariate and multivariate analyses of pretreatment variables were conducted using a Cox proportional hazards regression model to identify significant variables for disease-free survival (DFS) and overall survival (OS). Statistical analyses were performed using SAS version 9.3 (SAS Institute, Cary, NC) and R 2.13.2 (Vienna, Austria; http://www.R-project.org/). Time-dependent ROC curves were determined using the "survival ROC" package for R [21]. All tests were two-sided, and $P$ values <.05 were considered statistically significant.