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Original contribution

The use of an immunohistochemical diagnostic panel to determine the primary site of cervical lymph node metastases of occult squamous cell carcinoma☆

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Summary Cervical lymph node metastases from unknown primary sites account for approximately 3% to 9% of all head and neck malignant lesions. Squamous cell carcinoma is the most common type of cervical metastatic carcinoma. Our aim was to investigate the possibility of determining the site of primary tumors using an immunohistochemical diagnostic panel in metastatic cervical lymph nodes. Expression profiles of cytokeratins, 5/6; 8/18; 10; 13; 14; and 19, p16, and pRb were evaluated in 101 consecutive patients with cervical nodal metastasis who had undergone neck dissection to treat known head and neck squamous cell carcinoma (primary sites: 16, oral cavity; 38, oropharynx; 26, hypopharynx; 21, larynx). Cytokeratin 10 was more frequently expressed in oral cavity primary tumors, whereas cytokeratin 19 staining was more frequently observed in tumors originated from the pharynx and larynx. The expression of p16 and altered pRb status (0% or >50%) were more frequently observed in oropharynx primary tumors. To select the best subset among the 8 antibodies tested, classification and regression tree analysis was performed. The analysis correctly classified the four primary sites (25.0% of oral cavity, 89.5% of oropharynx, 30.8% of hypopharynx, and 57.1% of larynx) using 5 variables (histologic subtype, p16, cytokeratins 10 and 19, and pRb). The p16 was the single best predictor. The classification tree method using immunostaining profiles of p16, cytokeratins 10 and 19, or pRb may be helpful in the identification of the primary site of metastatic squamous cell carcinoma with occult primary.

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1. Introduction

A cervical lymph node metastasis can be the first clinical presentation of head and neck squamous cell carcinoma (HNSCC). In many cases, the primary site is identified by a subsequent extensive diagnostic workup that includes clinical, radiological, and endoscopic investigations; however, some primary lesions cannot be identified, even after a thorough clinical evaluation. Cervical lymph node metastases from unknown primary sites account for approximately 3% to 9% of all head and neck malignant lesions [1,2]. Squamous cell carcinoma (SCC) accounts for 70% to 90% of these lesions and most commonly arises from the upper aerodigestive tract [3].

In contrast to adenocarcinomas, which have relatively specific tumor markers, there are currently no useful immunohistochemical markers that can identify the original site of SCC.

When SCC is identified in a cervical lymph node and no primary site is identified, radical neck dissection and radiotherapy, or concurrent chemo-radiotherapy are usually applied, which results in increased morbidity. Therefore, it is important to identify the primary tumor to provide targeted therapy. Previous studies have shown that molecular methods such as microsatellite analysis or human papillomavirus (HPV) DNA detection were clinically useful tools for guiding the search for the sites of origin of these tumors [2,4]. However, these methods are often not available in daily diagnostic practice.

The cytokeratin (CK) is an intermediate filament protein found in the intracytoplasmic cytoskeleton of epithelia and their neoplasms. At least 20 different CKs have been described, and expression of these CKs is frequently tissue-specific and differentiation-specific [5]. In addition to being markers of normal epithelial differentiation, CK profiles have been used as a diagnostic tool to identify and classify different types of carcinomas.

The present study was conducted to investigate the possibility of determining the site of primary tumors using an immunohistochemical diagnostic panel in metastatic cervical lymph nodes.

2. Material and methods

2.1. Patients and samples

In this retrospective study, the surgical pathology records of all patients treated for cervical lymph node metastases from HNSCC at the Department of Otolaryngology–Head and Neck Surgery, Seoul St Mary's Hospital, The Catholic University of Korea, between 1996 and 2007 were reviewed. Cervical lymph node metastases from non-SCC were excluded from this study. We collected 101 consecutive patients with cervical nodal metastasis who had undergone neck dissection to treat known HNSCC.

2.2. Tissue microarray generation

Tissue microarrays were constructed from archival formalin-fixed, paraffin-embedded tissue blocks using a manual tissue arrayer (Quick-Ray Manual Tissue Microarrayer, Unitma Co, Ltd, Seoul, Korea). For each sample, areas rich in tumor cells were identified by light microscopic examination of hematoxylin-eosin–stained sections and then selected for use in tissue microarrays. Tissue cylinders with a diameter of 2 mm were punched from the previously marked tumor area of each block (donor block) and then transferred to a recipient paraffin block. This resulted in a 6 × 10 array for 60 cases. Duplicate tissue cores per specimen were arrayed on recipient paraffin blocks in order to decrease the error introduced by sampling and to minimize the impact of tissue loss during processing. The recipient paraffin block was then cut into 5-μm paraffin sections using standard techniques.

2.3. Immunohistochemistry

Immunohistochemical reactions were conducted on paraffin tissue sections using an automated immunohistochemical stainer (Lab Vision Autostainer LV-1; LabVision/Neomarkers, Fremont, CA) according to the manufacturer's protocols. Briefly, the tissue sections were deparaffinized and quenched with 3% hydrogen peroxide for 10 minutes. Antigen retrieval was then conducted using 0.01 mol/L citrate buffer (pH 6.0) by heating the sample in a microwave vacuum histoprocessor (RHS-1, Milestone, Bergamo, Italy) at a controlled final temperature of 121°C for 15 minutes. The primary antibodies (Table 1) were then diluted in Dako Antibody Diluent (Dako, Carpinteria, CA) with background-reducing components, after which they were incubated at room temperature for 30 minutes and then detected using the Envision plus System (Dako). The

Table 1 Sources and dilution of antibodies used

Antibody	Clone	Dilution	Source
CK 5/6	D5/16 B4	1:100	Dako
CK 8/18	Zym5.2 (UCD/PR-10.11)	1:200	Invitrogen, Camarillo, CA
CK 10	DEK-10	1:200	BioGenex, San Ramon, CA
CK 13	AE8	Ready to use	BioGenex
CK 14	LL002	1:50	BioGenex
CK 19	RCK108	1:50	Dako
p16	16P07	1:100	Neomarkers
pRb	polyclonal	1:100	Thermo Scientific, Cheshire, UK

Abbreviation: CK, cytokeratin.