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Original contribution

Frequent hepatocyte growth factor overexpression and low frequency of *c-Met* gene amplification in human papillomavirus–negative tonsillar squamous cell carcinoma and their prognostic significances ☆

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Summary Human papillomavirus (HPV) is an important prognostic factor for tonsillar squamous cell carcinoma (TSCC). HPV-positive and HPV-negative TSCCs are considered distinct in terms of prognosis and sensitivity to chemo/radiotherapy. However, to date, no study has thoroughly evaluated the individual prognostic factors for these 2 disease subgroups. Hepatocyte growth factor (HGF)-Met signaling pathway can be a predictive marker for prognosis or therapy response, especially in HPV-negative TSCC. We therefore investigated the prognostic values of HGF and c-Met expression in TSCC according to HPV status. Immunohistochemical analyses of HGF and c-Met protein expression and silver in situ hybridization of *c-Met* gene copy number were performed in 79 formalin-fixed, paraffin-embedded specimens. In HPV-negative TSCC, HGF overexpression, regional lymph node category, and ipsilateral cervical nodal metastasis predicted decreased overall survival (OS) ($P = .017$, $P = .024$, and $P = .003$, respectively). The latter 2 were also independent prognostic factors for progression-free survival ($P = .023$ and $P = .002$, respectively). In HPV-positive TSCC, heavy alcohol consumption and advanced primary tumor category were predictive of progression-free survival, whereas no independent prognostic factor for OS was identified. HGF overexpression had a significant effect on OS in HPV-negative TSCC but not in HPV-positive TSCC. HPV-negative/HGF-high expression tumors exhibited the worst survival outcomes, whereas HPV-positive/HGF-low expression tumors had the most favorable

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prognosis. c-Met expression and *c-Met* gene amplification were not associated with survival outcomes in TSCC patients. In conclusion, HGF may be a potential prognostic marker in HPV-negative TSCC, whereas c-Met exhibited limited clinical significance in TSCC.

1. Introduction

Tonsillar squamous cell carcinoma (TSCC), a highly aggressive malignancy with frequent local or regional recurrence and early lymphatic dissemination, is the most common oropharyngeal cancer, accounting for approximately 70% to 80% of all cancers of this area [1,2]. Conventional treatment for TSCC includes extensive surgery with or without radiotherapy and chemotherapy, depending on the disease stage and lymph node status [1,2]. Despite advances in surgical procedure and multimodality treatment, the survival of TSCC patients is dismal with a 5-year survival rate of 37% and a poor functional outcome [3,4]. For predicting the clinical behavior of TSCC, the presence of human papillomavirus (HPV) is an important factor indicating better prognosis and a higher response rate to chemo/radiotherapy [5,6]. Conversely, the absence of HPV infection may indicate worse patient survival and tumor resistance to chemo/radiotherapy, suggesting that HPV-negative TSCC patients would require more intense treatment for prolonged survival despite potential functional consequences [6]. Therefore, it is important to identify molecular markers that are predictive of therapy response and prognosis to stratify patients to more radical surgical approach or further adjuvant therapy, which ultimately reduces surgery-related morbidity and improves patient survival.

Hepatocyte growth factor (HGF)–Met signaling pathway plays an important role in the development and progression of head and neck squamous cell carcinoma (SCC) [7]. Overproduction of HGF and/or overexpression of c-Met by tumor cells or tumor-associated stromal cells contribute to the aberrant stimulation of this pathway [8]. HGF exerts mitogenic and motogenic effects on target epithelial and endothelial cells via its receptor c-Met [8]. The activation of this signaling pathway can trigger tumor cell migration, proliferation, and escape from apoptosis, resulting in the acquisition of invasive and metastatic phenotypes [7,8]. Hence, HGF and/or c-Met expression is associated with increased tumor aggressiveness and poor prognosis of cancer patients [9-14]. Therapy targeting this pathway could be effective in treating TSCC. Interestingly, it has been reported that an elevated plasma HGF level is a prognostic factor for worse overall survival (OS) in HPV-negative head and neck cancer patients receiving radiotherapy with cisplatin [15]. We therefore hypothesized that HGF or c-Met expression may play a prognostic role in HPV-negative TSCC. As the literature reporting clinical and pathologic data on HGF and/or c-Met as prognostic and predictive factors for HPV-negative TSCC is scarce, the present study was performed to address this clinically relevant question.

This study aimed to investigate the clinical or pathologic significance of HGF and c-Met expression in HPV-positive and HPV-negative TSCCs through immunohistochemical and silver in situ hybridization (SISH) analyses.

2. Materials and methods

2.1. Patients and histologic evaluation

The present study was conducted using formalin-fixed, paraffin-embedded tissues obtained from 79 patients with primary TSCC who underwent surgery at Ilsong Memorial Institute of Head and Neck Cancer, Kangdong Sacred Heart Hospital between 1997 and 2010. The selection criteria included the following patients: (1) those who underwent primary resection, (2) those with no prior treatment, and (3) those with available complete medical records, including pathologic slides and paraffin blocks of resected specimens. Clinical information was analyzed using medical records and radiologic study results. Smoking history was measured in pack-years, and patients were classified into 2 categories using 20 pack-years as the cut-off value, with heavy smoking defined by more than 20 pack-years [16]. Similarly, alcohol consumption was divided into 2 categories using 14 drinks/week as the cut-off value, and heavy alcohol consumption was defined by more than 14 drinks/week [16]. Surgical resection was followed by postoperative radiotherapy in 16 patients and chemo/radiotherapy in 34 patients. Twenty-nine patients were treated with surgery alone.

Histopathologic characteristics were independently reviewed by 2 pathologists. Diagnosis and histologic differentiation were evaluated according to the World Health Organization classification [17]. Staging was based on the American Joint Committee on Cancer staging system [18]. The tumor growth pattern at the invasive front was categorized as either pushing or infiltrative, with the former being a well-defined pushing margin with large tumor islands, and the latter consisting of scattered small irregular cords or single tumor cells with a poorly defined infiltrating margin [16]. Forty-five of the 79 tumor samples had been included in previously published studies [16,19]. This study was approved by the Institutional Ethics Committee of Kangdong Sacred Heart Hospital.

2.2. Tissue microarray and immunohistochemistry

Tissue microarray (TMA) was constructed for each paraffin block using a TMA manufacturing tool (Quick-