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# Expression of *c-Met* in laryngeal carcinoma

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**Abstract** Expression of *c-Met*, a gene for the hepatocyte growth factor/scatter factor (HGF/SF) receptor, is known to be associated with tumour development in several human carcinomas. The expression of *c-Met* was examined using immunohistochemistry in 82 cases of primary laryngeal carcinoma to evaluate the tissue distribution of *c-Met* and the clinicopathological significance of *c-Met* expression. In normal larynx, *c-Met* expression was observed only in some minor salivary glands. Positive reaction for *c-Met* in neoplastic epithelium was noted in 45 out of 82 (54.9%) cases. In 44 cases, structures adjacent to the carcinoma (noncancerous squamous epithelium, some stromal fibroblastic cells, and endothelial cells) showed positive reaction for *c-Met*. *c-Met* expression in cancerous epithelium was significantly correlated with lymph node status ($P<0.04$) and proliferating activity expressed by the Ki-67 labelling index ($P<0.02$). There was no correlation between *c-Met* expression and age, sex, histological type, T category, distant metastasis or clinical stage. The data suggest that overexpression of *c-Met* in laryngeal carcinomas represents a growth advantage for cancer cells, which may be conferred by the mitogenic effect of HGF/SF. Simultaneous *c-Met* expression in noncancerous components of the larynx may represent a paracrine modification of *c-Met.*

**Key words** c-Met · Ki-67 · Squamous cell carcinoma · Laryngeal carcinoma · Hepatocyte growth factor

## Introduction

The *c-Met* oncogene encodes an epithelial transmembrane tyrosine kinase receptor for hepatocyte growth factor/scatter factor (HGF/SF) [2]. As reported previously, HGF/SF has been designated a mitogen, a morphogen, and a motogen in various tumours [3, 17, 18, 21, 22, 25]. It has also been shown to act as a potent stimulator of angiogenesis [4,10]. Thus HGF and *c-Met* expression may have a multifactorial role in tumour progression by either paracrine or autocrine circuits [1, 6, 7, 13, 14, 28, 33]. Expression of *c-Met* in human adenocarcinomas is well described, but few studies on its expression in squamous cell carcinomas have been reported [18, 31]. We investigated expression and distribution of the *c-Met* oncogene in normal and cancerous laryngeal tissues by immunohistochemistry. Details of in vivo distribution of *c-Met* in normal and cancerous laryngeal tissues are described, and the correlation between oncogene expression and clinicopathological variables and the Ki-67 labelling index in laryngeal carcinomas is examined.

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## Materials and methods

Eighty-two patients seen for laryngeal carcinoma at the Saga Medical School Hospital were enrolled in this study between 1989 and 1996. Informed consent for the use of tissue for research purposes was obtained. Surgically removed or biopsy specimens of laryngeal squamous cell carcinoma, excluding carcinoma in situ and verrucous carcinoma, were used. None of the patients had undergone radiotherapy or chemotherapy before biopsy or surgical excision. The tumours were classified according to the 1987 UICC TNM classification system [11]. The average age at clinical onset was 67 years, with a range of 41–89 years. The types by locations were as follows: supraglottic (13), glottic (60), subglottic (5) and transglottic (4). Sixty-six patients had T1 or T2 disease and 16 patients, T3 or T4. Twelve patients had lymph node metastases. The histological typing was based on the World Health Organization (WHO) system [29]. Of the 82 tumours, 57 were well differentiated (G1), 17 moderately differentiated (G2), and 8 poorly differentiated (G3). Laryngeal carcinomas were divided into keratinizing (KT, 74 cases) and nonkeratinizing (NKT, 8 cases), the former including G1 and G2 tumours and the NKT, only the G3 tumours. The specimens were fixed in 10% formalin and embedded in paraffin, and 5-μm-thick sections were sliced and stained with haematoxylin and eosin (H&E). Normal post-mortem laryngeal tissue samples were obtained from 9 patients with no past history of laryngeal disease.

Immunohistochemical investigations were performed on the formalin-fixed paraffin-embedded tissue with a streptavidin—biotin kit (Nichirei Co., Tokyo, Japan) as described previously, with minor modification [5, 12, 25]. The primary antibodies used in this study were anti-*c-Met* polyclonal antibody (C-12; Santa Cruz Biotechnology, Santa Cruz, Calif) and monoclonal anti-Ki67 (MIB-1; Immunotech, Marseille, France). The peroxidase activity was revealed by 0.05% 3,3′-diaminobenzidine tetrahydrochloride (Sigma, St. Louis, Mo.) and haematoxylin was used for nuclear staining. For each experiment, negative controls omitting either primary or secondary antibodies were included to examine non-specific staining. The slides were reviewed by at least two observers and scored semiquantitatively as follows: – no staining, ± definite but weak staining, + moderate staining, and ++ strong staining. Tumour samples were scored positive if tumour cells were scored + or ++. The distribution of staining was characterized as focal (a few positive cells), part (groups of positive cells), or diffuse (sheet of positive cells).

To calculate the Ki-67 proliferative index, five carcinoma nests were arbitrarily chosen from the carcinoma lesion. Carcinoma cells and MIB-1-positive carcinoma cells were counted in these five nests, and the tumour proliferative fraction was expressed as the number of MIB-1-positive nuclei per 1000 cells.

Statistical comparisons were made using the two-tailed Student's *t*-test or the Chi-square test. *P*-values of <0.05 were regarded as significant.

## Results

In all normal laryngeal tissue, mild to moderate positive immunoreactivity for *c-Met* was observed in both acinar and ductal cells in the minor salivary glands (Fig. 1). Other constituent cells were not positive for *c-Met*.

Definite *c-Met* staining was noted in 45 out of 82 (54.9%) primary laryngeal cancers in the cytoplasm (Fig. 2), and in 10 out of 12 (83.3%) lymph node metastases and 3 out of 3 (100%) other metastases. When positive staining was observed, there was no significant intratumour heterogeneity from area to area. In lymph node metastases, *c-Met* immunoreactivity was observed in both the primary site and the metastatic foci in the lymph nodes in 9 out of 10 cases.

*c-Met* was expressed and detected moderately or strongly on elongated spindle-shaped cells lying within tumour stroma in 38 cases. These cells had a fibroblastic morphology (Fig. 2c). The minor salivary glands and the ducts located just beneath the tumour cells were moderately or strongly stained in 44 out of 60 cases (Fig. 2a). Weak to moderate *c-Met*-positive staining was observed in vascular endothelial cells adjacent to the tumour in 34 cases. *c-Met* also showed moderate immunoreactivity in noncancerous epithelium adjacent to the tumour in 33 cases (Fig. 2a).

c-Met staining was significantly correlated with lymph node status ($P<0.04$), although no correlation was detected between its expression and age, histological type, T category, distant metastasis or stage. The relation between expression of *c-Met* and clinical and histopathological parameters is summarized in Table 1.

The Ki-67 labelling index was not correlated with age, sex, tumour location, clinical stage, tumour grade and nodal or distant metastasis. The Ki-67 labelling index of laryngeal squamous cell carcinomas was significantly enhanced in comparison with that of normal laryngeal epithelium. MIB-1-positive nuclei were distributed randomly in the laryngeal carcinomas. Although cell-to-cell correlation between Ki-67 and c-Met positivity was not apparent on microscopic observation, *c-Met* positive carcinomas showed significantly higher Ki-67 labelling indices ($P<0.02$, Fig. 3) than negative tissues.

## Discussion

Hepatocyte growth factor/scatter factor (HGF/SF) and its receptor, *c-Met*, have been shown to fulfil a number of important roles in tumour progression, invasion and metastasis [7, 8, 13, 14, 21, 25, 26, 28, 30, 32, 34], and also in non-neoplastic processes [19, 20, 22, 35], such as acute gastric mucosal injury [33]. Its overexpression has

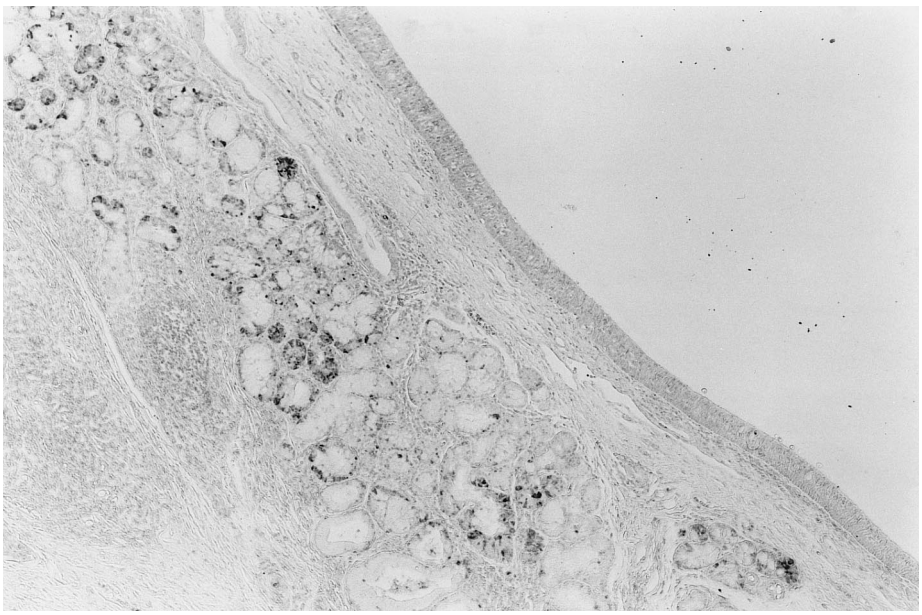

**Fig. 1** Immunostaining of c-Met in normal laryngeal tissue. c-Met positive cells are seen in some minor salivary glands. ×40

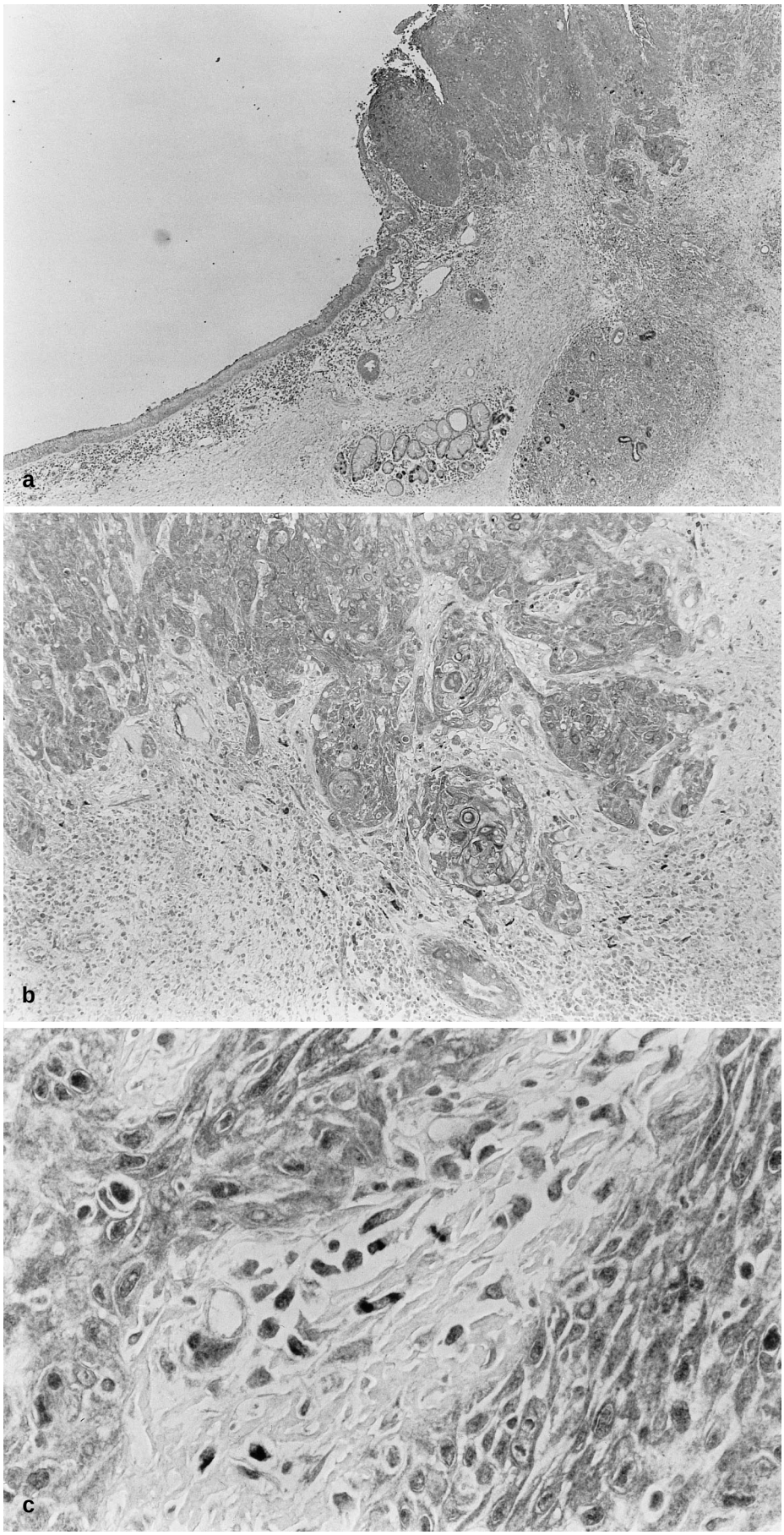


**Fig. 2a–c** Immunostaining of c-Met in laryngeal carcinoma. **a** c-Met is immunolocalized in squamous cell carcinoma, non-cancer epithelium adjacent to the cancer and minor salivary glands. ×40. **b** All cancer cells are positive for c-Met. ×125. **c** Spindle-shaped cells in the stroma are strongly stained. ×400

**Table** 1 Relationships between expression of c-Met and clinicopathological features in laryngeal carcinoma (*NKT* nonkeratinizing type, *KT* keratinizing type, *N.S.* not significant)

| Feature | Expression of c-Met | | *P* |
|---|---|---|---|
| | Negative | Positive | |
| Age (*n*=82) | | | |
| ≤67 | 21 | 28 | |
| >67 | 16 | 17 | N.S. |
| Histological type (*n*=82) | | | |
| KT | 35 | 39 | |
| NKT | 2 | 6 | N.S. |
| T category (n=82) | | | |
| T1/T2 | 32 | 34 | |
| T3/T4 | 5 | 11 | N.S. |
| Nodal status (*n*=82) | | | |
| pN0 | 35 | 35 | |
| pN+ | 2 | 10 | 0.032 |
| Distant metastasis | | | |
| M0 | 37 | 42 | |
| M1 | 0 | 3 | N.S. |
| Stage (*n*=82) | | | |
| I/II | 30 | 30 | |
| III/IV | 7 | 15 | N.S. |

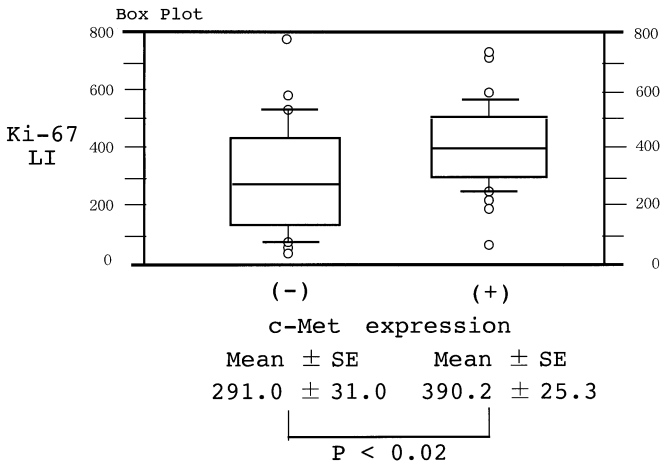


**Fig. 3** Ki-67 labelling index in c-Met negative (–) and *c-Met*-positive (+) laryngeal carcinomas (*LI* labelling index, *SE* standard error)

been reported in gastric [16], prostatic [13, 25], pancreatic [7, 8], breast [14, 21, 34], thyroid [6], and hepatocellular carcinomas [1, 28, 30]. Recent immunohistochemical in situ hybridization studies have shown that HGF/SF and *c-Met* are overexpressed simultaneously in breast and prostatic carcinomas, and the HGF/SF-c-Met receptor pair may have a role in the carcinomic progression in some tumours [14, 34]. This suggests that *c-Met* is activated by either a paracrine or an autocrine circuit and modulates the biological effects of SF/HGF upon a variety of neoplastic epithelial cell processes, such as cell motility, proliferation, invasiveness, and tubular morphogenesis. *c-Met* expression in squamous cell carcinoma has rarely been reported [18, 31] and *c-Met* expression in laryngeal carcinoma has not previously been reported.

More than half of the laryngeal carcinomas studied showed increased *c-Met* expression, whereas noncancerous squamous epithelium in control cases showed no *c-Met* reactivity. Clinicopathological indices, nodal status and Ki-67 index showed significant correlations with *c-Met* immunoreactivity. The Ki-67 antigen is expressed by non-G0 proliferating cells and has been widely used to assess cellular proliferative activity [9]. Although there is no direct evidence, an increased Ki-67 index in *c-Met*-positive carcinomas may be due to a mitogenic effect of HGF/SF and may represent a growth advantage in *c-Met*-positive carcinomas. Because both nodal status and proliferative activity are well-known indices for aggressive clinical behaviour in some squamous cell carcinomas [15, 23, 24, 27], *c-Met* receptor expression may also be important during the progression of laryngeal squamous carcinoma. Our data support the findings published in previous reports [13, 14, 25]. Our observations demonstrate that overexpression of *c-Met* occurred not only in carcinoma cells but also in stromal fibroblastic cells, endothelial cells and minor salivary glands and normal epithelium adjacent to the tumour. These observations are compatible with reports describing histological distributions of *c-Met*-positive cells in both neoplastic and non-neoplastic conditions [8]. Recent investigations concerning *c-Met* expression and HGF/*SF-Met* interaction have provided scientific evidence for the presence of up-regulation of Met receptor expression in both epithelial and stromal cells via autocrine and/or paracrine mechanisms [14, 34]. On the basis of what has been observed and reported about the distribution of *c-Met*-positive cells, it is conceivable that activated molecular signalling events of the HGF/SF-Met receptor system may involve both neoplastic cells and adjacent non-neoplastic epithelial and stromal cells. Our study provides the first demonstration of a correlation between *c-Met* expression and the clinicopathological indices of laryngeal carcinoma.

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