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Expression and Significance of C-Met Protein in Papillary Thyroid Carcinoma

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The c-met protein, encoded by the c-met oncogene and its ligand, the hepatocellular growth factor/ scatter factor, are known to be responsible for the motility and mitogenesis of epithelial cells including cancer cells. Recent studies have reported the prognostic significance of the c-met protein in malignant tumors. Papillary thyroid carcinoma, the most common histological type of thyroid carcinoma, can easily metastasize to regional lymph nodes, reflecting the activated motility and invasiveness of the carcinoma cells. We examined the expression of c-met protein in papillary thyroid carcinomas to assess its significance. Immunohistochemical staining of the c-met protein was performed on archival materials. The c-met protein was expressed in 10 cases of papillary thyroid carcinoma with recurrence, and in 5 of 10 cases without recurrence. Normal thyroids were negative for c-met protein. Expression of the c-met protein was statistically associated with recurrence of the thyroid carcinoma ($p = 0.016$). It is suggested that expression of the c-met protein plays a role in the recurrence of papillary thyroid carcinoma.

Key words : C-Met protein, Papillary thyroid carcinoma, Recurrence

INTRODUCTION

The c-met oncogene encodes transmembrane tyrosine kinase with certain structural features. The c-met protein is a 190 kDa heterodimer composed of two disulphide-linked chains, an extracellular 50 kDa α-chain, and a transmembrane 145 kDa β-chain [12], with tyrosine kinase activity [14]. The c-met protein arises from a single polypeptide precursor, which undergoes co- and post-translational glycosylation and endoproteolytic cleavage [11].

Hepatocyte growth factor (HGF) was first identified as a blood-derived mitogen for hepatocytes in culture as part of an effort to identify blood-borne hepatic mitogens arising during liver regeneration [13, 17]. Bottaro *et al*. reported that HGF induces tyrosine phosphorylation of the c-met protein, suggesting that HGF is a ligand [2]. Scatter Factor (SF) is a fibroblast-secreted protein which promotes motility and matrix invasion of epithelial cells. Scattering activity was originally observed in human mammary epithelial cells [23]. Both HGF and SF bind with identical affinities to the same sites in target cells. The receptor for HGF and SF was identified as the c-met protein by ligand binding and coprecipitation in immune complexes, chemical cross-linking to the β-subunits of c-met protein, transfer of binding activity in insect cells by a baculovirus carrying the c-met oncogene cDNA, and ligand-induced tyrosine phosphorylation of c-met β-subunits. HGF and SF cDNA clones from human fibroblasts, placenta, and liver had virtually identical sequences. Therefore, HGF and SF are thought to be indistinguishable ligands for c-met protein [18].

By analogy with other oncogene encoding tyrosine kinases, the c-met oncogene has

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been found to be involved in neoplastic disease [4, 19, 22]. Overexpression of the c-met oncogene has been demonstrated in several human tumors including papillary thyroid carcinoma (PTC) [6, 7, 20]. PTC is the most common histological type of thyroid carcinoma, accounting for 80 % of the cases. The incidence of regional lymph node metastasis in PTC ranges from 37 to 65 % [1], Active motility and invasiveness of the carcinoma cells. In this study we examined the expression of c-met protein in papillary thyroid carcinoma to assess its significance.

MATERIALS AND METHODS

Twenty cases of papillary thyroid carcinoma surgically removed in Ito Hospital from 1981 through 1994 were examined. Ten normal thyroid glands, obtained from surgical specimens unrelated to thyroid pathology, served as controls.

The 20 cases of PTC were divided into 2 groups, 10 cases with recurrence within 10 years (Table 1A) and 10 cases without recurrence during a follow-up of more than 10 years (Table 1B). Recurrence sites of papillary thyroid carcinoma included local sites such as the cervical lymph nodes, thyroid remnants, trachea, or muscles of the neck, and the lungs. Tumor-nodes-metastasis (TNM) classification of malignant tumors was performed according to standards of the International Union against Cancer [15]. All specimens were fixed in 10 % formalin and embedded in paraffin for routine pathological examination and diagnosis in Ito Hospital. Sections 3 μm thick were cut, mounted on adhesive-coated slides, deparaffinized with xylene, and hydrated in ethanol. Endogenous peroxidase was blocked by immersion in 0.3 % H_2O_2 in methanol for 30 min. The slides were placed in plastic jars containing 10 mM citrate buffer and heated in a H2800 microwave processor (Energy Beam Inc., MA) for 20 min to unmask the antigen. After cooling, the sections were placed in phosphate-buffered saline (PBS) containing 2 % (v/v) normal swine serum (DAKO, Denmark) for 10 min. The slides were then incubated at 4 ℃ overnight with rabbit polyclonal anti-c-met protein antibody (Santa Cruz, CA; 1 : 200 dilution), washed with PBS, and incubated for 30 min with biotinylated anti-rabbit IgG (Vector Laboratories, CA) as a second antibody. The slides were then incubated for 30 min with avidin-biotinyl-peroxidase complex (ABC) using a Vectastain ABC kit (Vector Laboratories). The peroxidase reaction was developed by placing the slides in 0.007 % (v/v) hydrogen peroxidase in Tris-HCl buffer (pH 7.6) for 5–10 min, using 0.02 % (w/v) 3.3' -diaminobenzidine tetrahydrochloride as the chromogen. The sections were counterstained with hematoxylin. The slides were washed three times with PBS between each step. As controls, tissue sections were incubated with normal rabbit immunoglobulin (DAKO) instead of the primary antibody, and yielded negative results in all cases.

Immunohistochemical staining was assessed semi-quantitatively as follows: more than 50 % of tumor cells staining positive (2 +), 10 to 50 % of tumor cells staining positive (1 +), scattered faint staining with less than 10 % of tumor cells positive (+ / −), and no staining (−). Grades of 2 + and 1 + were interpreted as positive for c-met protein. Statistical significance was evaluated either by Fisher' s exact probability test or the chi-squared test with a p value of more than 0.05 as the standard.

RESULTS

Clinical profiles of 10 PTC patients with recurrence and 10 PTC patients without recurrence are summarized in Table 1A and 1B. In the 10 PTC patients with recurrence, the mean age was 49.8, and the male to female ratio was 2 : 8. In the 10 PTC patients without recurrence, the mean age was 50.4, and the male to female ratio was 1 : 9. The 20 PTC patients consisted of 13 without regional lymph node metastasis (N0 by TNM classification) and seven with regional lymph node metastasis (N1 by TNM classification). None of the patients showed distant metastases at the time of initial diagnosis (M1 by TNM classification). In the 10 PTC patients with recurrence, 7 showed only local recurrence and 3 showed a distant metastasis, lung metastasis or local recurrence. All 20 patients with carcinomas were alive in 2000 even though local recurrence or lung metastasis had occurred.

Immunohistochemical findings of c-met protein are summarized in Table 2. Immunoreactivity of c-met protein antibody was demonstrated predominantly as cytoplasmic staining in tumor cells. All 10 primary

Table 1A PTC Patients with recurrence

Case.	Gender	Age	TNM *	c-met **	Recurrence free interval
1	F	35	T1N0M0	2 +	24
2	F	56	T3N0M0	2 +	125
3	F	56	T2N1M0	2 +	85
4	F	54	T3N1M0	2 +	18
5	M	69	T4N1M0	2 +	54
6	F	25	T3N0M0	2 +	71
7	F	47	T4N0M0	2 +	59
8	F	56	T2N1M0	2 +	45
9	F	33	T2N1M0	2 +	39
10	M	67	T2N1M0	2 +	11

* Tumor-Nodes-Metastasis
** Immunoreactivity

Table 1B PTC Patients without recurrence **

Case.	Gender	Age	TNM *	c-met **	Recurrence free interval
11	F	43	T1N0M0	−	114
12	F	58	T2N0M0	−	114
13	F	70	T2N0M0	−	113
14	F	56	T3N0M0	−	114
15	F	47	T2N0M0	−	114
16	F	58	T2N0M0	1 +	113
17	F	18	T2N0M0	1 +	113
18	F	55	T2N0M0	2 +	113
19	F	66	T3N0M0	2 +	113
20	M	33	T1N1M0	1 +	113

* Tumor-Nodes-Metastasis
** Immunoreactivity

Table 2 Regional lymph node recurrences and c-met products

Histologic grading	−	+ / −	1 +	2 +
with recurrence	0	0	0	10
without recurrence	4	1	3	2

specimens of PTC with recurrence strongly expressed c-met protein as 2+ grade positive (Fig. 1). In the 10 PTC patients without recurrence, 2 (20 %) and 3 (30 %) were 2 + and 1 + (Fig. 2) respectively. Overall, of the 20 PTC patients, 15 (75 %) were positive for c-met protein. In the 15 PTC patients positive for c-met protein, 10 showed recurrence and 5 did not. The expression of c-met protein in PTC was statistically correlated with recurrence ($p = 0.016$).

Association between regional lymph node metastasis and expression of c-met protein is summarized in Table 3. In 13 N0 patients, 8 were c-met protein positive (61.5 %) and 4 showed recurrence. All 7 N1 patients were c-met protein positive (100 %) and 6 had recurrences. All 7 cases of N1 patients were positive for c-met protein but the relation did not show statistical significance ($p = 0.083$).

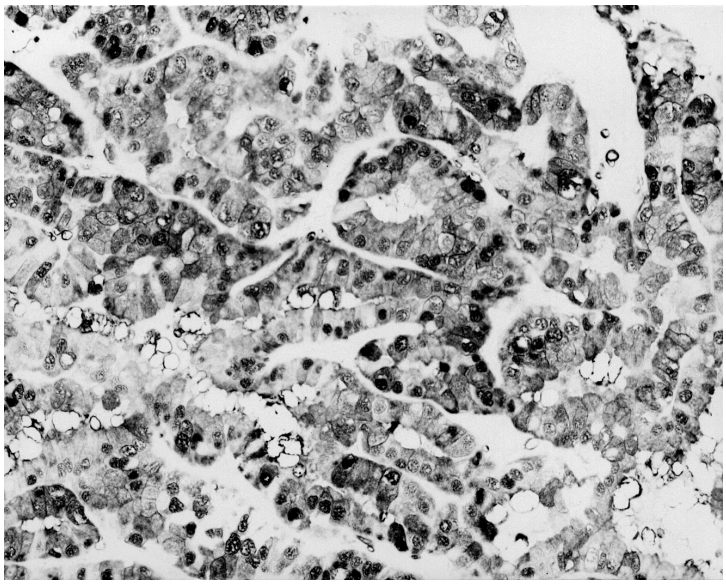

Fig. 1 Papillary thyroid carcinoma; 2 + immunoreactivity of c-met protein.

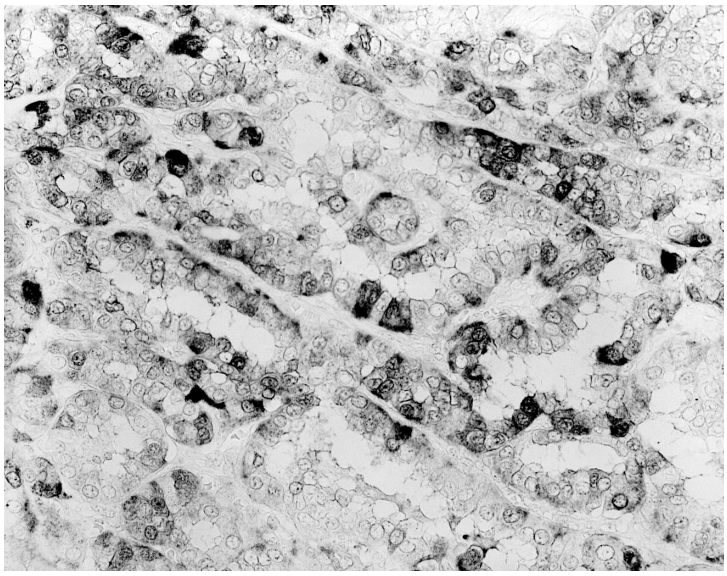

Fig. 2 Papillary thyroid carcinoma; 1 + immunoreactivty of c-met protein.

Table 3 Regional lymph node metastasis and c-met products

	No.	Number of patients positive for c-Met products
N0 *	13	8 (61.5%)
N1 **	7	7 (100%)

* N0 = Without Regional Lymph Node Metastasis
** N1 = With Regional Lymph Node Metastasis

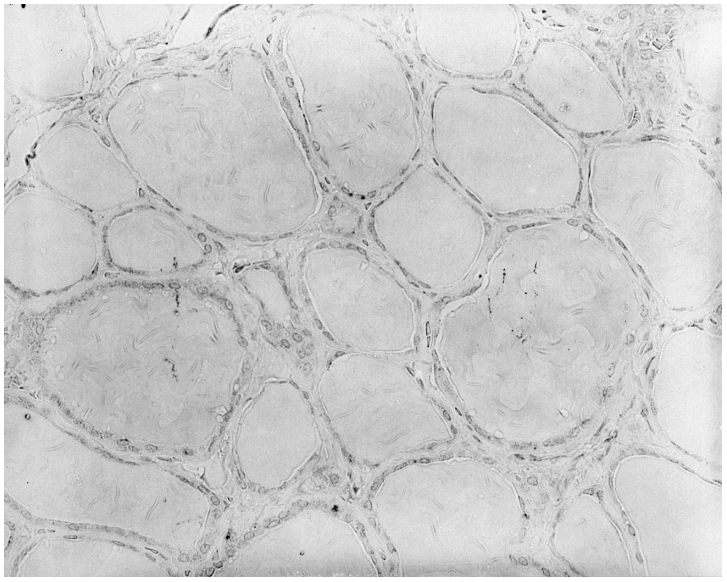

Fig. 3 Epithelial cells of normal thyroid are negative for c-met protein.

Normal thyroid tissue did not express c-met protein (Fig. 3).

DISCUSSION

In this study we composed papillary thyroid carcinomas with normal thyroid tissues. Fifteen of 20 PTC patients (75%) were positive for c-met protein. Seven PTC patients with regional lymph node metastasis at the time of initial diagnosis (N1) were positive for c-Met protein. Furthermore, the 10 PTC patients with recurrence were positive for c-met protein ($p = 0.016$), suggesting a close association between expression of the protein and recurrence. Our immunohistochemical analysis revealed that c-met protein was expressed more frequently both in PTC with regional lymph node metastases and with recurrences than in PTC without lymph node metastases and without recurrences. Thus, expression of c-met protein in PTC appears to be associated with the activated motility and invasiveness of cancer cells.

Staining for c-met protein was observed in the cytoplasm, in the perinuclear space, and in the Golgi area of neoplastic thyroid cells. Before the met protein is exposed to the plasma membrane, different maturation steps of the protein occur in succession in the endoplasmic reticulum and in the different compartments of the Golgi apparatus [5]. The c-met protein and its ligand, HGF, are known to have important stimulatory effects on the mitogenesis and motility of epithelial cells. The tissue distribution of c-met protein indicates that this molecule is involved in growth control of epithelial cells other than hepatocytes and suggests that its increased expression may confer a growth advantage on neoplastic cells [6]. Several studies have shown that overexpression of c-met protein is closely related to this growth advantage and to the pathological stage of the tumor in many carcinomas such as lung, hepatocellular, and pancreatic carcinomas [8, 16, 24]. Dremier *et al.* showed that HGF stimulated the proliferation and mobility of canine thyroid cells and suppressed the expression of differentiation markers, suggesting the possible role of c-met protein expression and its ligand, HGF, in human thyroid tumors [9]. The concomitant expression of c-met protein and HGF appeared to contribute to the growth and progression of carcinoma, in a paracrine manner, in the lungs and pancreas [10, 21]. Zarnegar *et al.* reported that thyroid parafollicular-C cells secrete HGF, and a paracrine relationship might also affect the aggressive behavior of thyroid tumors [25]. In this study we showed that expression of c-met protein was statistically associated with recurrence of PTC.

Coburn *et al.* reported that although recurrence rates of differentiated thyroid

carcinoma were higher in patients with regional lymph metastasis than in patients without such metastasis, the difference was not statistically significant [3]. We found that expression of c-met protein can be used as another predictor of recurrence in PTC. Even if patients with PTC do not have regional lymph node metastasis at the time of initial diagnosis, strict follow-up and observation are required if tests for c-met protein are positive.

In conclusion, immunohistochemical expression of the c-met protein is higher in PTC patients with recurrences than without recurrences, indicating that expression of c-met protein plays a pathological role in the recurrence of PTC.

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