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Overexpression of c-Met/hepatocyte growth factor receptors in human prostatic adenocarcinoma.

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Abstract

Hepatocyte growth factor (HGF) and c-met proto-oncogene product (c-Met) have varied biological functions in different tissues and have been implicated in mitogenic, motogenic and morphogenic responses in both organ regeneration and carcinogenesis. Some studies have suggested that the overexpression of c-Met and epidermal growth factor receptor (EGFR) are associated with growth advantage, while transforming growth factor-beta receptor II (TGF beta R II) is associated with growth disadvantage of human prostatic adenocarcinoma. However, it is unclear if the expression of c-Met correlates with the expression of EGFR and TGF beta R II, and with the proliferative status of human prostatic adenocarcinoma. Using immunohistochemical staining with anti-c-Met (C-12), anti-EGFR (NCL-EGFR) and anti-TGF beta R II (L-21) antibodies, we determined the frequency of expression of c-MET, EGFR, and TGF beta R II respectively in a series of 134 radical prostatectomy specimens. We evaluated the relationship between the expression of these receptors and clinicopathological characteristics. Overall, c-Met immunostaining was detected in 54 of 134 (40.3%) cases, EGFR in 45 (33.6%) and TGF beta R II in 64 (48.4%). The overexpression of c-Met was significantly more common in poorly differentiated ($P < 0.0001$) and in the diffusely infiltrated specimens ($P < 0.0005$). In contrast, TGF beta R II was significantly overexpressed in the well differentiated specimens ($P < 0.0001$) and associated negatively with c-Met ($P < 0.0001$). Overall, these data suggest that c-Met/HGF receptor and TGF beta R II overexpression may be involved in the differentiation of human prostatic adenocarcinoma, c-Met with de-differentiation and TGF beta R II with differentiation.

KEYWORDS: c-met proto-oncogene product, epidermal growth factor receptor, transforming growth factor- β receptor ?, prostatic adenocarcinoma, immunohist chemistry

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Overexpression of c-Met/Hepatocyte Growth Factor Receptors in Human Prostatic Adenocarcinoma

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Hepatocyte growth factor (HGF) and c-met proto-oncogene product (c-Met) have varied biological functions in different tissues and have been implicated in mitogenic, motogenic and morphogenic responses in both organ regeneration and carcinogenesis.

Some studies have suggested that the overexpression of c-Met and epidermal growth factor receptor (EGFR) are associated with growth advantage, while transforming growth factor- β receptor II (TGF β R II) is associated with growth disadvantage of human prostatic adenocarcinoma. However, it is unclear if the expression of c-Met correlates with the expression of EGFR and TGF β R II, and with the proliferative status of human prostatic adenocarcinoma. Using immunohistochemical staining with anti-c-Met (C-12), anti-EGFR (NCL-EGFR) and anti-TGF β R II (L-21) antibodies, we determined the frequency of expression of c-MET, EGFR, and TGF β R II respectively in a series of 134 radical prostatectomy specimens. We evaluated the relationship between the expression of these receptors and clinicopathological characteristics. Overall, c-Met immunostaining was detected in 54 of 134 (40.3%) cases, EGFR in 45 (33.6%) and TGF β R II in 64 (48.4%). The overexpression of c-Met was significantly more common in poorly differentiated ($P < 0.0001$) and in the diffusely infiltrated specimens ($P < 0.0005$). In contrast, TGF β R II was significantly overexpressed in the well differentiated specimens ($P < 0.0001$) and associated negatively with c-Met ($P < 0.0001$). Overall, these data suggest that c-Met/HGF receptor and TGF β R II overexpression may be involved in the differentiation of human prostatic adenocarcinoma, c-Met with de-differentiation

and TGF β R II with differentiation.

Key words: *c-met* proto-oncogene product, epidermal growth factor receptor, transforming growth factor- β receptor II, prostatic adenocarcinoma, immunohistochemistry

In human cancers, activation and amplification of endogenous proto-oncogenes have been shown to play important roles in biological mechanisms.

Hepatocyte growth factor (HGF) and its receptor, the *c-met* proto-oncogene product (c-Met), have varied biological functions. They have been implicated in mitogenic (1-3), motogenic (2-4), and morphogenic (5) responses during organ regeneration. Hepatocyte growth factor and c-Met have also been implicated in tumor growth suppression (6, 7) and carcinogenesis (8). c-Met was found to be overexpressed in normal and neoplastic human tissues such as stomach, colon, pancreas and thyroid (8-10) and shown to correlate with cancer differentiation and proliferation (9-10). In human prostatic cancer, a significant correlation between c-Met expression and grade has previously been reported (11, 12). However, it is unclear if c-Met expression correlates with the expression of any other growth factor receptor such as epidermal growth factor receptor (EGFR) and transforming growth factor- β receptor II (TGF β R II). In addition, the relation of these receptors with the proliferative index has yet to be determined in prostatic carcinoma.

The incorporation of EGF and EGFR promotes proliferation and development of ectodermal, mesodermal and endodermal cells (13), and is also involved in embryogenesis, cellular differentiation and angiogenesis (14).

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EGF and transforming growth factor- α (TGF- α) share 35 percent sequence homology, and they are both ligands for EGFR. They have been reported to have very similar biological properties (13, 14) in human breast (15, 16), gastric (17), and colon (17) carcinomas.

TGF- β is involved in a several biological processes such as mitogenesis, morphogenesis and inhibition of tissue differentiation (18). It has also been demonstrated that TGF β R II expression in gastric (19) and colon cancer (20) correlates with the degree of sensitivity of these cancers to growth inhibition by TGF- β .

A few studies have demonstrated that growth advantage or disadvantage in the development of human prostatic adenocarcinoma is associated with the expression of

c-Met (12, 21) and EGFR (22, 23), or TGF β R II (19–20). The purpose of this study is to determine whether c-Met expression is associated with the expression of EGFR or TGF β R II and clinicopathological characteristics in human prostatic carcinoma.

Materials and Methods

Tumor samples. Samples were drawn from a total of 134 cases of human prostatic adenocarcinomas (median patient age 68.3; range 48–80 years) between 1984 and 1996 at the Department of Urology, Kochi Medical School, Division of Urology, Kochi-Takasu Hospital and Chikamori Hospital, Kochi, Japan, were

Table 1 The relationships between the expression of c-Met, epidermal growth factor receptor (EGFR) or transforming growth factor β receptor II (TGF β R II) and each clinicopathological factor

Clinico-pathological factors	c-Met		EGFR		TGF β R II		Total
	Neg.	Pos.	Neg.	Pos.	Neg.	Pos.	
Age (years) 70 >	44	32	49	27	38	38	76
70 \leq	36	22	40	18	32	26	58
pT 1	13	4	12	5	1	16	17
2	25	19	30	14	24	20	44
3	40	27	43	24	41	26	67
4	2	4	4	2	4	2	6
DIF well	44	9	36	17	11	42	53
moderate	34	28	38	24	41	21	62
poor	2	17	15	4	18	1	19
INF α	21	3	11	13	7	17	24
β	40	22	40	22	32	30	62
γ	19	29	38	10	31	17	48
int 1	17	18	21	14	23	12	35
2	38	23	42	19	33	28	61
3	25	13	26	12	14	24	38
Total	80	54	89	45	70	64	134

Statistical significance:

c-Met \times DIF, TGF β R II \times DIF; $P < 0.0001$

c-Met \times INF; $P < 0.0005$

Clinicopathological factors:

pT: Depth of cancer cell penetration

1: Microscopical cancer foci; 2: Localized within prostate; 3: Penetration over prostatic capsule; 4: Invasion to adjacent organs

DIF: Type of cancer cell differentiation

well: Well-differentiated; moderate: Moderately-differentiated; poor: Poorly-differentiated

INF: Type of cancer cell infiltration

α : Expansive, well-defined pattern; β : Intermediate, moderately-defined pattern; γ : Diffusely invasive, ill-defined pattern

int: Amount of interstitium in cancer foci

1: Small amount of interstitium; 2: Intermediate amount of interstitium; 3: Large amount of interstitium

Categories of immunostaining:

Neg.: the case of cancer cells with negative or less than 75% positive staining

Pos.: the case of cancer cells with more than 75% positive staining

studied using immunohistochemistry. The patients' clinicopathological characteristics are shown in Table 1. All tumor specimens were fixed in 10 % buffered formalin, processed in the usual fashion, and embedded in paraffin. In each case, hematoxylin and eosin-stained sections were reviewed, and a representative block was chosen for further studies.

Immunohistochemistry. Each specimen was stained using a polyclonal antibody to c-Met (c-Met, C-12, dilution 1:50, Santa Cruz Biotechnology, Inc., USA), a monoclonal antibody to EGFR (EGFR, NCL-EGFR, 1:20, Novocastra Laboratories, Ltd., UK) and polyclonal antibody to TGF β R II (TGF β R II, L-21, 1:100, Santa Cruz Biotechnology, Inc.). Expression was assessed by immunohistochemical examination (streptavidin-biotin complex procedure) as reported previously (24). Each specimen was considered as c-Met-, EGFR-, or TGF β R II-overexpression when the defining staining of the cancer cell membrane was increased in comparison to normal prostate tissues. Each specimen was assigned to two categories depending on the number of positive cancer cells as follows: negative (Neg, less than 75 % of positive cancer cells) or positive (Pos, more than 75 % of positive cancer cells).

Statistical analysis. Clinical and pathological characteristics of tumors including age, depth of cancer cell penetration (pT), type of cancer cell differentiation (DIF), type of cancer cell infiltration (INF) and amount of interstitium in cancer foci (int) were determined for each tumor according to the "General Rule for Clinical and Pathological Studies on Prostatic Cancer" (25).

The correlations between expression of c-Met, EGFR, or TGF β R II, and these clinicopathological factors were analyzed statistically using the chi-square test at the 5 % level.

Results

c-Met positive immunostaining was detected in 54 of 134 (40.3 %) cases (Fig. 1a, b). Positive immunostaining for EGFR was detected in 45 (33.6 %) cases. In all positive cases, the cell membranes were homogeneously stained. Sixty-four of 134 (48.4 %) cases were positive for TGF β R II, including only 3 cases which were positive for both c-Met and TGF β R II (Fig. 2). There were no positive cases for TGF β R II which were also showed overexpression of c-Met and EGFR.

In adjacent normal and hyperplastic epithelium of

prostatic glands, weak immunoreactivity for c-Met and EGFR was restricted to the basal cells, and immunoreactivity for TGF β R II was detected at the luminal surface of the epithelium (data not shown).

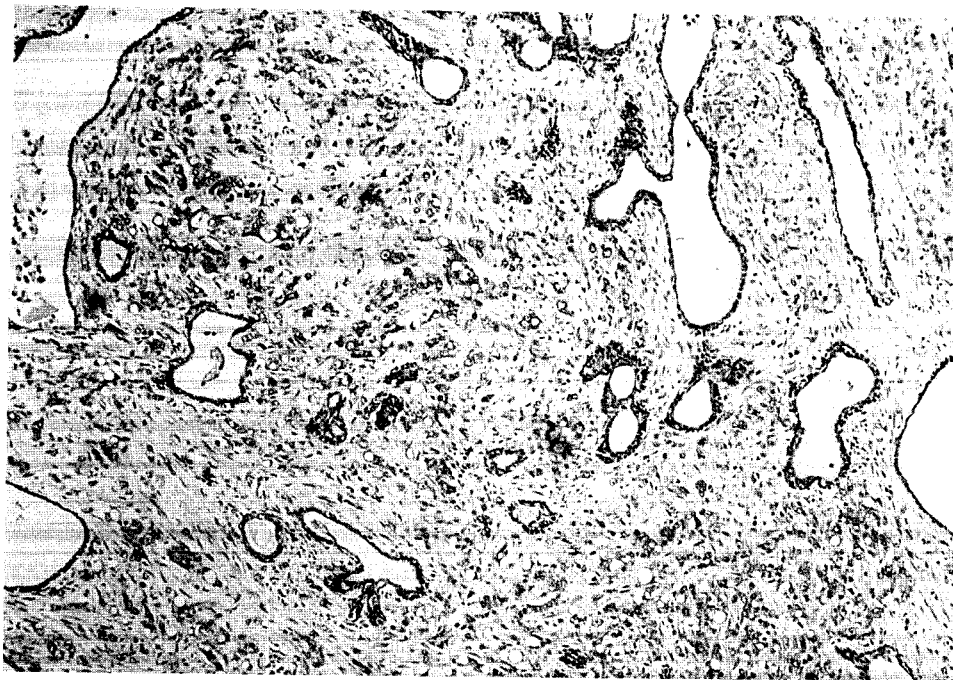
Table 1 shows the relationships between the expression of c-Met, EGFR, or TGF β R II and each clinicopathological factor.

The overexpression of c-Met was detected in 89.5 % (17/19) of the poorly differentiated cases and in 60.4 % (29/48) of diffusely infiltrating cases, which were statistically significant at $P < 0.0001$ and $P < 0.0005$ respectively. In contrast, overexpression of TGF β R II was detected in 79.2 % (42/53) of the well differentiated cases. Only one poorly differentiated case stained positive for TGF β R II. The correlation between TGF β R II expression and the level of differentiation was statistically significant ($P < 0.0001$). There was no statistical correlation between the expression of EGFR and any of the clinicopathological factors. Although no significant correlation was found between the expression of c-Met and EGFR, the overexpression of c-Met was significantly associated with the overexpression of TGF β R II in inverse proportion in prostatic adenocarcinoma (Table 2).

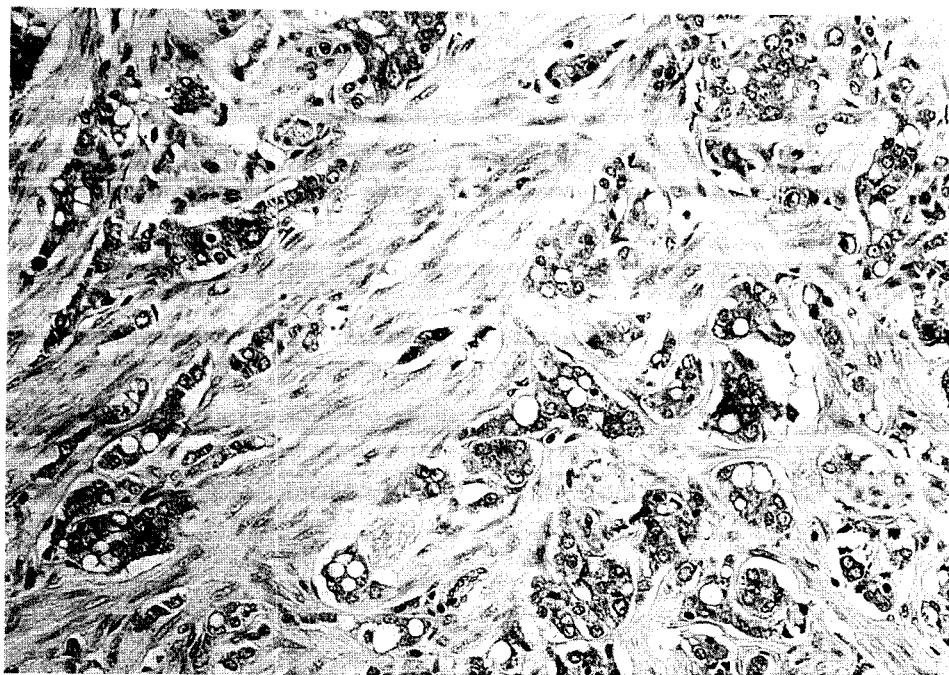
Discussion

In the present study, we used immunohistochemical techniques to demonstrate the correlation between the expression of c-Met, EGFR, and TGF β R II and clinicopathological factors in radical prostatectomy specimens.

We found that c-Met was overexpressed in 54 (40.3 %) of 134 cases. This result confirms recent studies reporting that c-Met was overexpressed in 33 % (2/6 cases) (21) or 84 % (36/43 cases) (12) of prostatic cancer. This overexpression of c-Met is thought to be secondary to gene amplification (8, 26). In this study we did not find any correlation between c-Met expression and tumor stage as previously reported (12, 21). However we did find that overexpression of c-Met statistically correlated with cancer differentiation ($P < 0.0001$) and diffuse infiltration ($P < 0.0005$). Eighty-nine percent of the poorly differentiated tumors showed overexpression of c-Met. Similar results were reported in pancreatic (9) and thyroid (10) carcinomas. Although it is not elevated in thyroid adenoma and anaplastic adenocarcinoma, the expression of c-Met was increased 100-fold increased in thyroid papillary carcinoma (10). In that study, the authors suggested that overexpression of c-Met may confer to thyroid



a



b

Fig. 1 The overexpression of c-Met is confined to the cytoplasm of the cancer cells in a cases of poorly-differentiated (a) and diffusely infiltrating human prostatic adenocarcinoma (b). (Streptavidin-biotin complex method, a; $\times 250$, b; $\times 500$).

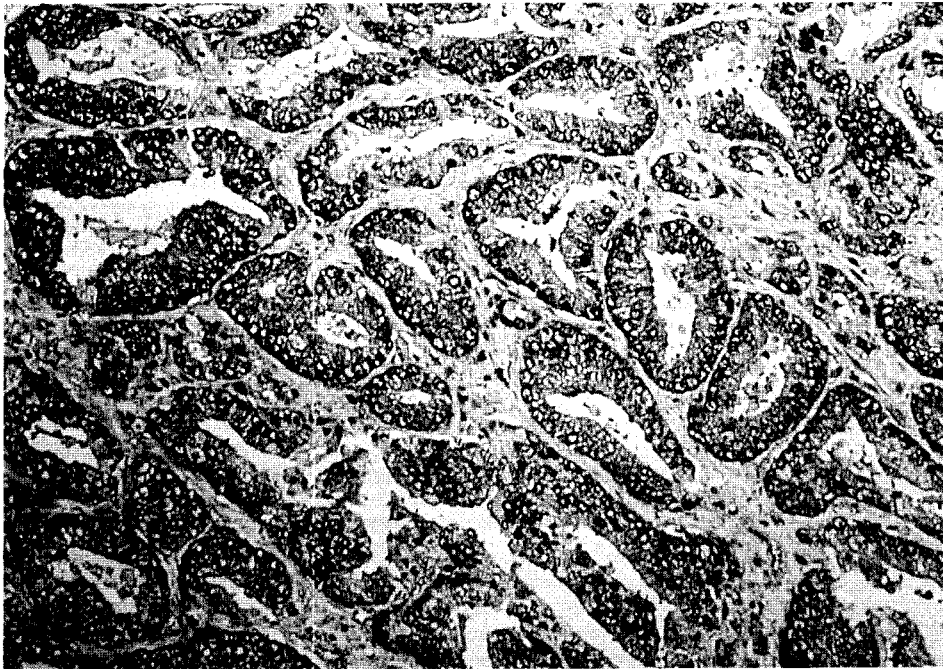


Fig. 2 The overexpression of TGF β R II is detected only in the cytoplasm of the cancer cells in a case of well-differentiated prostatic adenocarcinoma. (Streptavidin-biotin complex method, $\times 500$)

Table 2 The relationships between the expression of c-Met, EGFR and TGF β R II

		c-Met		TGF β R II		Total
		Neg.	Pos.	Neg.	Pos.	
c-Met	Neg.	—	—	19	61	80
	Pos.	—	—	51	3	54
EGFR	Neg.	44	45	42	47	89
	Pos.	36	9	28	17	45
Total		80	54	70	64	134

Statistical significance: c-Met \times TGF β R II; $P < 0.0001$.

Neg.: Pos.: See legend to Table 1.

carcinomas the ability to progress towards more advanced disease through the acquisition of a more aggressive phenotype (10). In our study, we found that the overexpression of c-Met is strongly associated with poorly differentiated prostatic adenocarcinoma and may play a significant role in the growth and progression of human prostatic adenocarcinoma.

Although EGFR was detected in 45 of 134 (33.6%) cases, including only 9 in which c-Met was also positive,

there were no statistically significant relationships between EGFR expression and any of the clinicopathological factors studied. These results confirm recent studies in which it was reported that 17% (5/19 cases) (22) or 40% (41/102 cases) (23) of prostatic cancer expressed EGFR, with no significant correlation to clinicopathological factors. However, we observed that all the cases in which c-Met and EGFR were expressed were poorly differentiated, suggesting that expression of both growth factor receptors may contribute to the poorly differentiated phenotype.

Furthermore, the overexpression of TGF β R II was clearly associated with well differentiated tumors ($P < 0.0001$) and that this expression was inversely proportional to that of c-Met. It is possible that a balance between these growth factor receptors may play a role in tumor progression.

In summary, although the biological significance of c-Met remains in question, our report demonstrates that c-Met overexpression correlates with the poorly differentiated phenotype and is inversely proportional to TGF β R II expression in a large series of patients with prostatic adenocarcinoma. An imbalance between c-Met and

TGF β R II expression may play a role in early differentiation toward a more malignant phenotype and early progression of human prostatic cancer. Further studies are needed to determine if the expression of these molecular markers correlate with prognosis and could be used to identify high risk patients.

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