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Overexpression of *c-met* proto-oncogene associated with chromophilic renal cell carcinoma with papillary growth

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Abstract Various genetic changes are involved in human renal cell carcinomas (RCCs). However, the molecular events related to other cytomorphological subtypes of RCC are not well known, apart from the relationship between the von Hippel-Lindau tumour suppressor gene and clear cell subtype RCC. We examined the overexpression of several growth factor receptors immunohistochemically and analyzed their relationship to the cytomorphological characters in 120 cases of RCCs. These receptors included *c-met* proto-oncogene product (c-MET), epidermal growth factor receptor (EGFR) and transforming growth factor beta receptor II (TGFβR). The overexpression of c-MET was detected in all cases (20/20) of the tubulo-papillary growth type and 78.3% (18/23) of chromophilic cell subtype, resulting in a very significant associations between c-MET overexpression and tubulo-papillary growth RCCs ($P<0.0001$), c-MET and chromophilic subtype RCCs ($P<0.0001$), and c-MET and EGFR ($P<0.0001$). EGFR overexpression was significantly associated with the compact growth RCCs (49/89, $P<0.0001$), clear cell subtype RCCs ($P<0.005$) and the overexpression of TGFβR ($P<0.0001$). These results strongly suggest a close correlation between the overexpression of c-MET and development of the chromophilic subtype of RCC with papillary growth pattern. EGFR expression is closely related to the pathogenesis of the clear cell subtype of RCC with compact growth pattern. The overexpression of c-MET, EGFR, and TGFβR may have roles that are individually significant in the morphogenesis of RCC.

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Introduction

Renal cell carcinoma (RCC) is the most common malignant neoplasm in the kidney and mortality records on RCC indicate an increasing incidence. It has been reported that a number of genetic alterations, activation of proto-oncogenes or inactivation of tumour suppressor genes are involved in the pathogenesis of human RCCs [12, 22, 23]. Previous results show that inactivation of the VHL suppressor gene is strongly involved in the development of the clear cell subtype of RCC [22]. Each cytomorphological subtype of human RCC is reported to have its own genetic change in oncogenes or tumour suppressor genes [12, 23].

HGF and its receptor, the c-MET, have varied biological functions in different tissues and have been implicated in mitogenic [5, 9, 17], motogenic [4, 9], and morphogenic [10] responses and tumour suppression [21, 25] in tissue or organ regeneration and carcinogenesis [2]. In noncancerous disease of kidney many types of peptide growth factors have been detected, including c-MET [2]. A few earlier studies suggest that c-MET, EGFR, or TGFβR expression is related to the specific growth patterns of RCC [11, 19, 24, 27]. However, little is known about whether the expression of c-MET correlates with the receptors of other growth factors, such as EGFR and TGFβR, and with the morphological and cytological features of cancer cell proliferation in human RCC.

EGFR promotes proliferation and development of ectodermal, mesodermal and endodermal cells [14] and is also involved in embryogenesis, cellular differentiation and angiogenesis [8]. EGFR and transforming growth factor-α (TGFα) share 35% sequence homology, so they have very similar biological properties [8, 14]. EGFR and TGFα signals pass through the same EGFR, as observed in human breast [15, 18], gastric [28] and colonic [28] carcinomas.

TGFβ has multi-functional biological properties, as mitogen, morphogen or inhibitor in different tissues and organs [16]. It has been demonstrated that TGFβR expression in gastric [13] and colonic [7] cancer correlates with the degree of sensitivity of these cancer cells to growth inhibition by TGFβ.

We examined the expression of these growth factor receptors and compared the morphological and cytological features of RCCs. Our results suggest a close correlation between c-MET expression and the chromophilic subtype of RCC with papillary growth pattern, and between EGFR and the clear cell subtype of RCC with a compact growth pattern.

Materials and methods

One hundred and twenty cases of RCC obtained by radical nephrectomy between 1984 and 1996 at the Department of Urology of Kochi Medical School were studied by immunohistochemistry (IHC). Table 1 shows the clinicopathological characteristics of these 120 cases in patients age 41–81 (median age 57.2) years. All tumour specimens were fixed in 10% buffered formalin, processed routinely, and embedded in paraffin. In each case, all the available haematoxylin and eosin-stained sections were reviewed, and a representative block was chosen for further studies.

Table 1 Clinicopathological characteristics of 120 cases of renal cell carcinomas (*Grade* grade of nuclear atypism: *1* low grade, *2* intermediate grade, *3* high grade, *pT* depth of cancer cell penetration: *1* tumour is less than 2.5 cm and localized within kidney, *2* tumour over 2.5 cm and localized within kidney, *3* penetration through renal capsule, but within Gerota's fascia, *4* invasion of adjacent organs, *INF* type of cancer cell infiltration: α expansive, well-defined pattern, β intermediate, moderately-defined pattern, γ diffusely invasive, ill-defined pattern)

Clinicopathological factors		Total
Age (year-old)	<60	49
	60≤	71
Sex	Male	89
	Female	31
Cytological elements	Clear	90
	Chromophilic	23
	Chromophobe	0
	Spindle-shaped/ pleomorphic variants	7
	Oncocytic	0
Histological elements	Compact	89
	Tubulo-papillary	20
	Cystic	11
Grade	1	62
	2	50
	3	8
pT	1	6
	2	87
	3	26
	4	1
INF	α	69
	β	46
	γ	5
Total		120

Each specimen was assessed by IHC examination (streptavidin-biotin complex procedure) as reported previously [6], using polyclonal antibody to c-MET (c-MET c-12, dilution 1:50, Santa Cruz Biotechnology, USA), monoclonal antibody to EGFR (EGFR, NCL-EGFR, 1:20, Novocastra Laboratories, UK) and polyclonal antibody to TGFβR (TGFβR II, L-21, 1:100, Santa Cruz Biotechnology). Each specimen was considered to show c-MET, EGFR, or TGFβR overexpression when the definite positivity of the membrane of cancer cells with these antibodies was higher than in normal kidney. A visual assessment of the number of positive cancer cells was made as a population of the total expression of c-MET, EGFR, TGFβR as follows: negative (Neg.; cancer cells with negative staining or cancer cells with less than 50% positive staining), positive (Pos.; cancer cells with more than 50% positive staining). Each specimen also was immunostained without the each first antibody and was assessed as negative control.

Clinical and pathological classification of tumours including age, sex, cytological elements, histological elements, grade, depth of cancer cell penetration (pT) and type of cancer cell infiltration (INF) was performed according to the classification by Thoenes et al. [26] and the General Rule for Clinical and Pathological Studies on Renal Cell Carcinoma [20].

The correlations between the expression of c-MET, EGFR, TGFβR and the clinicopathological factors considered were analysed statistically using the Chi-square test at the 5% level.

Results

Overall, c-MET immunostaining was detected in 20 (16.7%) of the 120 cases. Immunostaining was confined mainly to the membrane of cancer cells in positive-staining cases (Fig. 1A). Table 2 shows the relationships between the frequency of overexpressed c-MET, EGFR of TGFβR and each clinicopathological factor. When these results were compared with cytomorphological features of RCCs, overexpression of c-MET was detected in 100% (20/20) of the tubulo-papillary growth type and 78.3% (18/23) of the chromophilic cancer cells, revealing a markedly significant association ($P<0.001$).

Positive immunostaining with EGFR antibody was detected in 54 of the 120 (45.0%) cases. All of the positive immunoreaction was found in the cell membranes of cancer cells (Fig. 1B). The overexpression of EGFR was detected in 55.1% (49/89) of compact growth type and 53.3% (48/90) of clear cell tumours (Fig. 1C). This receptor expression was significantly associated with compact growth type ($P<0.0001$) and clear cell tumours ($P<0.005$).

Forty-four of the 120 (36.7%) cases were positive for cell membrane staining with TGFβR antibody. However, there was no statistical relationship between the expression of TGFβR and any clinicopathological factor.

None of the cases tested showed a double positive reaction with c-MET and EGFR or c-MET and TGFβR. Twelve cases were doubly positive with EGFR and TGFβR. There were significant correlations between c-MET and EGFR and between EGFR and TGFβR ($P<0.0001$).

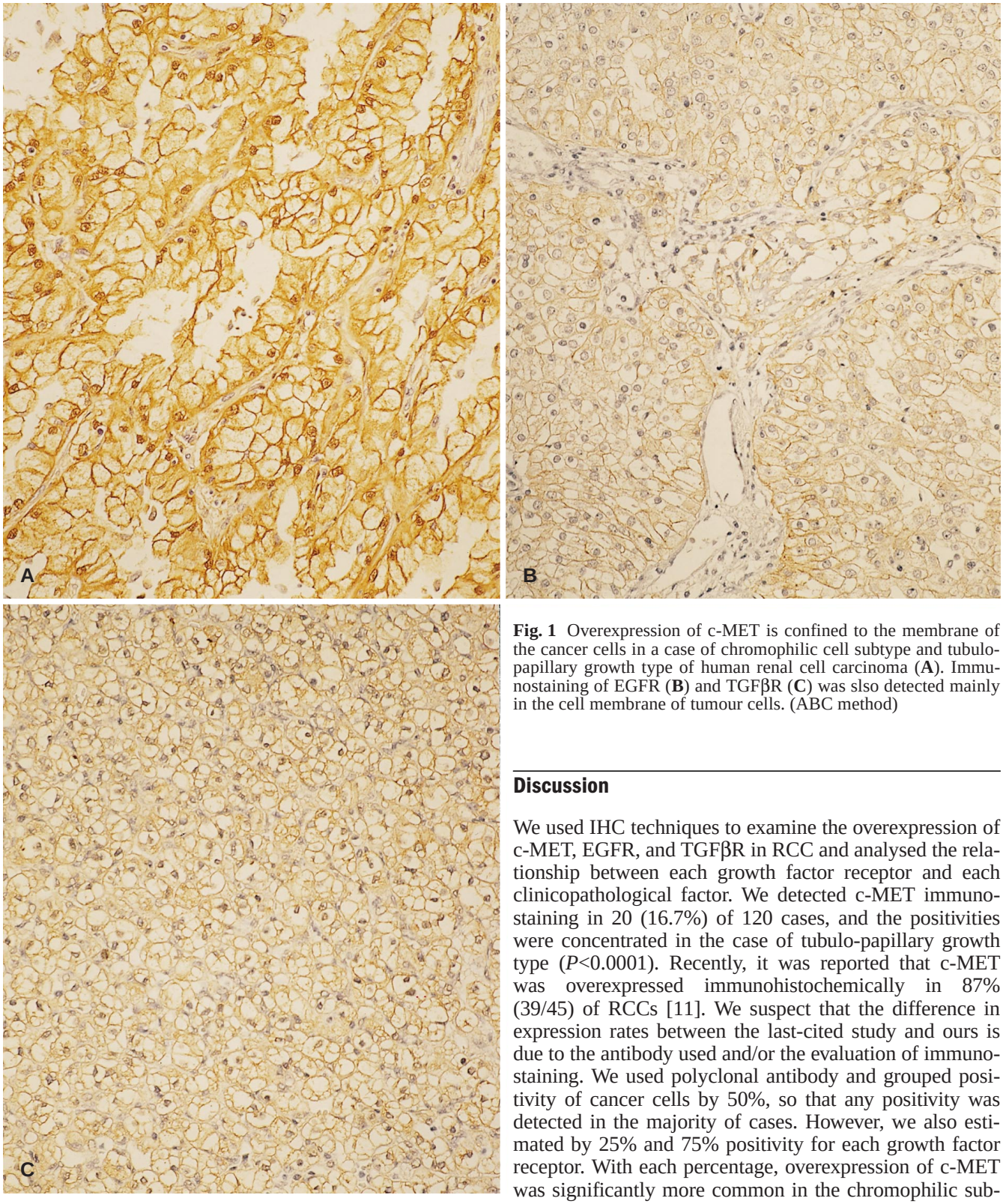

Fig. 1 Overexpression of c-MET is confined to the membrane of the cancer cells in a case of chromophilic cell subtype and tubulo-papillary growth type of human renal cell carcinoma (**A**). Immunostaining of EGFR (**B**) and TGFβR (**C**) was slso detected mainly in the cell membrane of tumour cells. (ABC method)

In adjacent normal tissues and adenomas of kidney, the immunoreactivity for c-MET, EGFR and TGFβR was lower than in cancer cells; the weak expression of c-MET and EGFR was restricted to the proximal tubules, and that of TGFβR to the proximal and distal tubules.

Discussion

We used IHC techniques to examine the overexpression of c-MET, EGFR, and TGFβR in RCC and analysed the relationship between each growth factor receptor and each clinicopathological factor. We detected c-MET immunostaining in 20 (16.7%) of 120 cases, and the positivities were concentrated in the case of tubulo-papillary growth type ($P<0.0001$). Recently, it was reported that c-MET was overexpressed immunohistochemically in 87% (39/45) of RCCs [11]. We suspect that the difference in expression rates between the last-cited study and ours is due to the antibody used and/or the evaluation of immunostaining. We used polyclonal antibody and grouped positivity of cancer cells by 50%, so that any positivity was detected in the majority of cases. However, we also estimated by 25% and 75% positivity for each growth factor receptor. With each percentage, overexpression of c-MET was significantly more common in the chromophilic subtype ($P<0.0001$) of RCC with papillary growth pattern ($P<0.0001$) and was not associated with the other clinicopathological factors. Therefore we evaluated cases where cancer cells had more than 50% positivity as positive.

Previous studies suggested that the six pathologically classified subtypes of human RCC [26] might each have

Table 2 The relationships between the expression of c-MET, EGFR of TGFβR and each clinicopathological factor (*Neg*.negative or less than 50% positive staining, *Pos*. cancer cellswith more than 50% positive staining)

Clinicopathological factors		c-MET		EGFR		TGFβR		Total
		Neg.	Pos.	Neg.	Pos.	Neg.	Pos.	
Age (year-old)	<60	41	8	27	22	31	18	49
	60≤	59	12	39	32	45	26	71
Sex	Male	73	16	48	41	55	34	89
	Female	27	4	18	13	21	10	31
Cytological elements								
Clear		88	2	42	48	50	40	90
Chromophilic		5	18	20	3	21	2	23
Spindle / pleomorphic		7	0	4	3	5	2	7
Histological elements								
Compact		89	0	40	49	49	40	89
Tubulopapillary		0	20	20	0	20	0	20
Cystic		11	0	6	5	7	4	11
Grade	1	56	6	34	28	37	25	62
	2,3	44	14	32	26	39	19	58
pT	1,2	77	16	52	41	61	32	93
	3,4	23	4	14	13	15	12	27
INF	α	59	10	38	31	44	25	69
	β,γ	41	10	28	23	32	19	51
Total		100	20	66	54	76	44	120

Statistical significance (P<0.0001): c-MET × Cytological elements, c-MET × Histological elements, EGFR × Histological elements, c-MET × EGFR, EGFR × TGFβR

(*Grade* grade of nuclear atypism; *1* low grade, *2, 3* : *2* or 3, *pT* Depth of cancer cell penetration, *1.2* 1 or 2, *3.4* : 3 or *4* (3 ; penetration through renal capsule within Gerota's fascia, 4 ; invasion to adjacent organs) β, γ : β or γ

their own pathogenetic mechanisms; abnormal expression of VHL tumour suppressor gene in the oncogenesis of the clear cell subtype has been reported [22], as have the loss of heterozygosity (LOH) (specific loss of chromosomes 1, 2, 6, 10, 13, 17 and 21) in the chromophobe subtype [23] and p53 gene mutation in the sarcomatoid transformation of RCC [12]. Schmidt et al. [19] identified the *c-met* proto-oncogene at chromosome 7q31.2 as the oncogene for the hereditary papillary renal cell carcinoma (HPRC) gene, where missense mutations located in the *c-met* proto-oncogene lead to constitutive activation of the c-MET protein and HPRCs [19]. The c-MET gene may be one of the activated oncogenes that lead to both sporadic papillary RCC and hereditary papillary RCC via an individual oncogenetic pathway.

There are a few reports that c-MET overexpression is associated with the onset and progression of RCC [11]. However, in this study, significant relationships between c-MET overexpression and tumour grade or stage were not demonstrated. In other cancers, such as pancreas [3] and thyroid [2] cancers, overexpression of c-MET has also been reported to be associated with cytomorphologic features of cancer cell proliferation resulting in progression. C-MET was overexpressed in papillary carcinomas derived from the follicular epithelium of thyroid glands, but was not expressed in non-neoplastic thyroid diseases, adenomas, or anaplastic carcinomas [2]. We also revealed c-MET expression in papillary carcinomas derived from proximal tubules, but not in adjacent normal tissue and papillary adenomas of kidney or any of several other cancers, such as cancers of the pancreas [3] and thyroid [2]. We therefore agree that c-MET overexpression may confer on carcinomas the ability to progress towards malignancy through the acquisition of a more aggressive behaviour [2].

Positive cases with EGFR were detected in 54 of our 120 cases (45.0%), mainly in the clear cell subtype (P<0.005) and compact growth type (P<0.0001) of RCC. The VHL gene is inactivated in most RCCs of the clear cell subtype. It has been reported that inactivation of the VHL gene results in the overproduction of several proteins, such as vascular endothelial growth factor (PDGFβ chain). Since our results have shown a correlation between the clear cell subtype of RCC and overexpression of EGFR, another pathway opened up by inactivation of VHL gene might be increased transcription of EGFR mRNA. It has been reported that overexpression of EGFR was found by IHC in 92% (32/34) [24] and 47% (7/15) [27] of cases of RCC, with significant correlations with clinicopathological factors.

Twelve double-positive cases with EGFR and TGFβR immunostaining showed no significant correlation with clinicopathological factors. These double overexpressions seemingly exert no additive influence on cancer cell differentiation and progression.

Although it has been reported that there is a correlation between the overexpression of TGFβR and cancer development in other human cancers, including gastric [13] and colonic [7] cancer, overexpression of TGFβR showed no significant correlations with the clinicopathological factors in RCC.

The molecular basis for positive immunostaining of c-MET remains under investigation. This is the first report of frequent c-MET overexpression in relation to other peptide growth factor receptors and suggests that overexpression of c-MET in RCC is involved in tubulo-papillary growth phenotype, possibly as a morphogenic factor. However, EGFR may be involved in the compact growth phenotype and as a morphogenic factor in RCC. More comprehensive studies involving greater numbers of cases of RCC and including measurement of DNA and/or RNA levels will be necessary to determine whether increased c-MET expression, alone or in combination with other genes, contributes to the cytomorphogenesis of RCC.

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