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Granulocyte macrophage colony-stimulating factor as a predictor of the response of metastatic renal cell carcinoma to tyrosine kinase inhibitor therapy

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Abstract. This prospective study was conducted to identify predictive markers for the response of metastatic renal cell carcinoma (RCC) to tyrosine kinase inhibitors (TKIs). Patients with histologically proven RCC with at least one measurable metastatic lesion were enrolled in this study. Blood samples were collected prior to treatment and the plasma levels of 27 cytokines were measured. Tumor response was assessed 8-12 weeks after the initiation of TKI treatment. A total of 13 patients (11 men and 2 women) with a median age of 63 years received sunitinib (8 cases), sorafenib (1 case), or axitinib (4 cases). Partial response (PR) was achieved in 5 patients (38%), stable disease (SD) in 4 (30%) and progressive disease (PD) was noted in 4 (30%). The plasma granulocyte macrophage colony-stimulating factor (GM-CSF) level in PR cases was significantly higher compared to that in SD or PD cases (P=0.012). Therefore, GM-CSF may be a predictive biomarker of the response of RCC to TKI treatment, suggesting that TKIs may exert clinical effects not only through suppression of the vascular endothelial growth factor, but also through immune system modulation.

Introduction

Renal cell carcinoma (RCC) is one of the major causes of cancer-related mortality. There were an estimated ~64,700 new cases of RCC and 13,570 deaths in 2012 in the United States (1). Over the last few years, a number of tyrosine kinase inhibitors (TKIs) have been proven to be effective and are currently widely used for the treatment of metastatic RCC. However, the effect of these TKIs appears to be rather limited, with only 31% of naive cases exhibiting an objective response [complete response (CR) or partial response (PR)] to sunitinib treatment in the first-line setting (2) and only 10% of cases with previous cytokine therapy exhibiting a PR to treatment with sorafenib (3). However, thus far, only a limited number of factors that predict the response of RCC to TKIs have been reported. A significant decrease in serum vascular endothelial growth factor (VEGF) receptor-2 levels and/or an increase in serum VEGF levels were observed in patients exhibiting an objective tumor response (4,5). Hypothyroidism and hypertension associated with TKI treatment were also reported to be correlated with a favorable response (6,7).

Although previous studies suggested that TKIs may affect the immune system (8,9), only a limited number of studies have investigated immunological biomarkers for therapeutic prediction. Adotevi *et al* (10) reported that a decrease in regulatory T cells was correlated with a favorable overall survival in cases with metastatic RCC who received sunitinib-based antiangiogenic therapy. Thus, we conducted a prospective study to invesigate predictive immunological biomarkers.

Patients and methods

Patients. Patients with histologically proven RCC with at least one measurable metastatic lesion, who were diagnosed between March, 2012 and June, 2013, were enrolled in this study. Sunitinib, sorafenib or axitinib were administered orally as previously described (2,3,11). Tumor response was assessed 8-12 weeks after the initiation of TKI treatment according to the response evaluation criteria in solid tumors and was classified as CR, PR, stable disease (SD) or progressive disease (PD) (12).

We collected blood samples from the 13 patients prior to treatment. The plasma was deep frozen at -80˚C and stored before measuring the immune function.

Cytokines. A total of 27 cytokines including interleukin (IL)-1β, IL-1ra, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9,

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Table I. Correlation between the clinical effect of tyrosine kinase inhibitors (TKIs) and clinicopathological characteristics among patients with metastatic renal cancer.

Clinical characteristics	Total (n=13)	Clinical effect[a] PR (n=5)	SD (n=4)	PD (n=4)	P-value
Gender					
Male	11	4	4	3	0.603
Female	2	1	0	1	
Age (years)					
≥65	7	2	2	3	0.593
<65	6	3	2	1	
Performance status					
0	8	3	2	3	0.780
1	5	2	2	1	
Laterality					
Right	8	2	3	3	0.479
Left	5	3	1	1	
Nephrectomy					
Radical	11	4	4	3	0.603
Partial	2	1	0	1	
Histology					
Clear cell RCC	11	5	2	4	0.085
Papillary RCC	2	0	2	0	
Nuclear grade					
G1/G2	12	4	4	4	0.449
G3	1	1	0	0	
Stage					
pT1	6	3	1	2	0.593
pT2/pT3/pT4	7	2	3	2	
Lymphovascular invasion					
0	2	1	1	0	0.603
1	11	4	3	4	
Lung metastasis					
No	3	1	2	0	0.267
Yes	10	4	2	4	
Bone metastasis					
No	8	2	4	2	0.180
Yes	5	3	0	2	
TKIs					
Sunitinib	8	4	3	1	0.219
Others	5	1	1	3	
Dose intensity (%)					
100	7	2	2	3	0.593
<100	6	3	2	1	
Previous treatment					
No	2	1	1	0	0.603
Yes	11	4	3	4	
Previous TKI treatment					
No	8	4	3	1	0.219
Yes	5	1	1	3	
Previous cytokine treatment					
No	5	3	1	1	0.479
Yes	8	2	3	3	

Table I. Continued.

Clinical characteristics	Total (n=13)	Clinical effect[a] PR (n=5)	SD (n=4)	PD (n=4)	P-value
Previous mTOR inhibitor treatment					
No	10	4	3	3	0.980
Yes	3	1	1	1	

[a]Best response during the 3-month treatment. The P-values were calculated using the Kruskal-Wallis test. PR, partial response; SD, stable disease; PD, progressive disease; RCC, renal cell carcinoma; mTOR, mammalian target of rapamycin.

IL-10, IL-12, IL-13, IL-15, IL-17, eotaxin, basic fibroblast growth factor, granulocyte colony-stimulating factor, granulocyte macrophage colony-stimulating factor (GM-CSF), interferon-γ (IFN-γ), IFN-γ-induced protein 10, monocyte chemoattractant protein-1, macrophage inflammatory protein (MIP)-1α, platelet-derived growth factor (PDGF)-BB, MIP-1β, regulated on activation, normal T-cell expressed and secreted, tumor necrosis factor-α and VEGF were measured twice by BioPlex Pro Human Cytokine 27 Plex assay (M50-0KCAF0Y; Bio-Rad, Hercules, CA, USA). The assay was performed according to the manufacturer's instructions. Briefly, plasma was centrifuged at 15,000 x g for 10 min at 4˚C. The samples were then incubated with microbeads labeled with specific antibodies to one of the aforementioned cytokines for 60 min. Following a washing step, the beads were incubated with the detection antibody cocktail, with each antibody specific to a single cytokine, for 30 min. After another washing step, the beads were incubated with streptavidin-phycoerythrin for 10 min, washed again and the concentration of each cytokine was determined using the array reader. The samples were tested in duplicate on a 96-well plate alongside the standard curve used to generate the results. Unknown concentrations were calculated from a standard curve generated from Bio-Rad supplied standards.

Statistical analysis. The correlation between clinical and cytokine data was analyzed by analysis of variance (ANOVA) and Tukey-Kramer's test using JMP software, version 10.0.0 (SAS, Institute, Cary, NC, USA).

This study was approved by the Institutional Ethics Committee of the Faculty of Medicine and Graduate School of Medicine of the University of Tokyo (no. H22-23-400).

Results

Patient characteristics. A total of 13 patients (8 treated with sunitinib, 1 with sorafenib and 4 with axitinib), including 11 men and 2 women, with a median age of 63 years (range, 50-77 years), were recruited in this study (Table I). The performance status was 0 in 8 and 1 in 5 cases. Eight tumors were located in the right and 5 in the left kidney. Radical nephrectomy was performed in 11 and partial nephrectomy in 2 patients. Histologically, the tumors were diagnosed as 11 clear cell RCCs and 2 papillary RCCs. All the patients had developed metastasis, with the most common metastatic site being the lung (10 cases), followed by bone (5 cases).

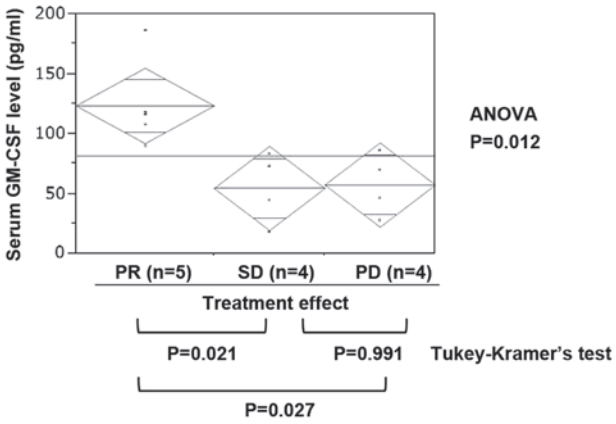

Figure 1. Comparison of serum granulocyte macrophage colony-stimulating factor (GM-CSF) levels among patients who achieved partial response (PR), stable disease (SD) or exhibited progressive disease (PD) after treatment with tyrosine kinase inhibitors. ANOVA, analysis of variance.

Treatment. Two cases received TKI treatment as first-line therapy. Previous systemic treatment included TKIs in 5, mammalian target of rapamycin (mTOR) inhibitors in 3 and cytokines in 8 patients. PR was achieved in 5 cases (38%), SD in 4 (30%) and PD developed in 4 cases (30%). The dose was reduced in 6 patients (46%) due to adverse events.

GM-CSF plasma levels by treatment response. No clinical parameters exhibited a significant correlation with treatment effect (Table I). Among the 27 investigated cytokines, the plasma GM-CSF level in PR cases was significantly higher compared to that in cases with SD or PD (Fig. 1, ANOVA, P=0.012; Tukey-Kramer's test: PR vs. SD, P=0.021; PR vs. PD, P=0.027; and SD vs. PD, P=0.991). The IL-6 level was higher in PD cases, but the difference was not statistically significant (Table II, P=0.141).

Discussion

We demonstrated that plasma GM-CSF may be a predictive marker of the response to TKI treatment. Thus far, only a few studies demonstrated the clinical utility of GM-CSF. The

Table II. Correlation between the clinical effect of tyrosine kinase inhibitors and cytokine levels in patients with metastatic renal cancer.

	Clinical effect			
Cytokines	PR	SD	PD	P-value
GM-CSF	123±36	54±29	57±25	0.012
IL-1β	3.8±4.6	1.9±1.2	1.6±0.2	0.494
IL-1ra	103±98	57±43	60±22	0.536
IL-2	5.2±2	4.8±3.1	5±3	0.971
IL-4	5.6±2.3	4.8±1.8	5.1±2.2	0.864
IL-5	1.1±1.4	0.7±0.8	0.6±0.8	0.791
IL-6	6±2.3	5±2.6	12±8.9	0.141
IL-7	5.3±1.9	4.8±4.9	3.1±2.2	0.605
IL-8	25±13	29±33	19±12	0.779
IL-9	44±12	26±8.8	29±15	0.125
IL-10	4.2±2.9	3.4±1.1	6.4±5.7	0.525
IL-12	19±17	17±15	32±37	0.658
IL-13	5.2±3.9	5.1±3.1	5.1±2.4	0.990
IL-15	5±1.6	3.6±2.3	4.2±0.6	0.479
IL-17	56±15	41±16	59±41	0.613
Eotaxin	183±152	128±126	112±61	0.667
FGF-basic	51±14	42±12	55±22	0.554
G-CSF	66±19	52±18	59±13	0.527
IFN-γ	610±893	207±94	178±21	0.462
IP-10	2,381±1,857	1,386±749	1,906±1,432	0.616
MCP-1	82±68	41±16	47±22	0.388
MIP-1α	2.9±1.1	7.7±12	2.9±1.7	0.561
PDGF-BB	309±306	862±146	213±128	0.508
MIP-1β	178±43	174±141	128±79	0.703
RANTES	3,364±138	2,630±763	2,679±771	0.523
TNF-α	88±92	62±47	43±6.9	0.580
VEGF	108±62	122±79	165±143	0.683

The results are expressed as mean ± standard deviation (pg/ml) and the P-values were calculated using analysis of variance. PR, partial response; SD, stable disease; PD, progressive disease; GM-CSF, granulocyte macrophage colony-stimulating factor; IL, interleukin; FGF, fibroblast growth factor; G-CSF, granulocyte colony-stimulating factor; IFN-γ, interferon-γ; IP-10, IFN-γ-induced protein 10; MCP-1, monocyte chemoattractant protein-1; MIP, macrophage inflammatory protein; RANTES, regulated on activation, normal T-cell expressed and secreted; PDGF, platelet-derived growth factor; TNF-α, tumor necrosis factor-α; VEGF, vascular endothelial growth factor.

plasma GM-CSF level was found to be higher in cervical cancer patients compared to healthy controls (13), while in another study GM-CSF was undetectable in non-cancer patients (14).

GM-CSF promotes the differentiation and expansion of myeloid-derived suppressor cells (MDSCs) (15,16). Antigen-specific $CD8^+$ T-cell tolerance, induced by MDSCs, is known to be one of the main mechanisms of tumor escape (17). Knockdown of GM-CSF in tumor cells may reverse the cytotoxicity to CD8 T lymphocytes: Dolcetti *et al* (15) found that lack of GM-CSF release from 4T1 mammary carcinoma cells reduced the accumulation of Gr-$1^{int/low}$ MDSC subsets and successfully inhibited tumor-induced tolerance in mice. Similarly, Serafini *et al* (16) demonstrated that inhibition of MDSC function abrogates the proliferation of regulatory T cells and tumor-induced tolerance in antigen-specific T cells, using the A20 B-cell lymphoma model *in vitro* and *in vivo*. However, TKIs may reduce the number of MDSCs in the tumor and normalize T-lymphocyte function: Xin *et al* (18) demonstrated that sunitinib directly induced RCC tumor cell apoptosis through Stat3 inhibition, which was accompanied by a reduction in MDSCs and tumor-infiltrating regulatory T cells.

These reports suggest that high levels of plasma GM-CSF may promote the function of MDSCs and escape of tumor cells from the host immune system. In patients with high GM-CSF levels, TKIs may decrease the function of MDSCs that is upregulated by GM-CSF and reverse the cytotoxicity of regulatory T lymphocytes directly or indirectly, which may lower tumor-induced tolerance and result in favorable treatment effects.

In our study, VEGF was not found to be significantly associated with treatment effect, contrary to previous reports (4,5). GM-CSF was reported to induce VEGF release from the epithelium, resulting in the promotion of carcinogenesis: Wang *et al* (19) demonstrated that, in a colitis-associated cancer model, blocking GM-CSF activity *in vivo* significantly decreased epithelial release of VEGF and abrogated cancer formation. In the plasma, GM-CSF, which is upstream of VEGF, may be a more sensitive biomarker for metastatic RCC treatment compared to VEGF.

As regards other biomarkers, Tran *et al* (20) screened pretreatment cytokines and angiogenic factors in patients with metastatic RCC who received pazopanib treatment and found that high IL-6 was predictive for unfavorable progression-free survival. In our study, IL-6 was also higher in PD cases, but the difference was not statistically significant.

This study had certain limitations. First, this was a single-institution study; and second, our sample size was limited.

In conclusion, high pre-treatment plasma levels of GM-CSF, which is an inducer of immune tolerance, were significantly associated with a favorable response of metastatic RCC to TKI treatment. The result suggests the potential of GM-CSF as a predictive biomarker of the response to TKI treatment. However, further investigation is required to determine the effects of TKIs on abrogating cancer immune tolerance.

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