Genetic Polymorphisms of the *Interleukin-4 Receptor* α Gene Are Associated with an Increasing Risk and a Poor Prognosis of Sporadic Renal Cell Carcinoma in a Japanese Population[1]

Eijiro Nakamura,[2] Yuzuru Megumi,[2] Takashi Kobayashi, Toshiyuki Kamoto, Satoshi Ishitoya, Toshiro Terachi, Mitsuhiro Tachibana, Hisanori Matsushiro, Tomonori Habuchi, Yoshiyuki Kakehi, and Osamu Ogawa[3]

Department of Urology, Kyoto University Graduate School of Medicine, Kyoto 606-8507, Japan [E. N., Y. M., T. Ko., T. Ka., S. I., T. T., Y. K., O. O.]; Laboratory of Anatomic Pathology, Kyoto University Hospital, Kyoto 606-8507 [M. T., H. M.]; and Department of Urology, Akita University School of Medicine, Akita 010-8543 [T. H.], Japan

ABSTRACT

Purpose: **It has been suggested that the immune system of the host may be capable of modulating the clinical course of renal cell carcinoma (RCC) patients. In fact, the amount of Th2 cytokines such as interleukin (IL)-4 and IL-10 in the serum of patients has been found to be an important predictor of poor prognosis. Recently, it was reported that genetic polymorphisms of the *IL-4 receptor* α (*IL-4R*α) gene affect the strength of signaling through the receptor. In addition, these same polymorphisms were found to be associated with an increased risk of atopy by causing Th2-dominated responses of the host. The significance of the polymorphisms on the incidence and prognosis in sporadic RCC patients were examined to clarify the role of IL-4 as well as that of the Th1/Th2 immune system in this disease.**

Experimental Design: **A case-control study was performed with 143 sporadic RCCs in a Japanese population and 205 Japanese controls. Logistic regression models were also used to assess the genetic effects on prognosis.**

Results: **The frequencies of variant alleles that enhance signaling of IL-4 were significantly related to an increased risk of RCC. Furthermore, multivariate regression analysis showed that the genotype of the *IL-4R* gene was an independent prognostic factor for cause-specific survival ($P = 0.018$) together with M classification ($P = 0.0002$) and histopathological grade ($P = 0.044$).**

Conclusions: **The present findings show that the preferential Th2-type response to tumors was associated with a poorer prognosis and suggest that polymorphisms of the *IL-4R*α gene may serve as useful genetic markers for assessing the risk of the development and progression of RCC.**

INTRODUCTION

RCC[4] has peculiar characteristics such as late relapses and spontaneous regressions of metastatic lesions after the resection of primary tumors. In addition, immunotherapy with IFN-α, IL-2, or a combination therapy of these biological response modifiers is the mainstay of management of metastatic RCC patients. These findings indicate a potential role for the host immune system in regulating tumor growth of RCC (1, 2). In support of this hypothesis, our previous study showed a significant association between *HLA-DRB1* polymorphisms and the risk of RCC (3).

Recently, considerable evidence has accumulated to suggest the existence of two types of responses in the human immune system: Th1 and Th2. Th1 cells produce IL-2, IFNs, and IL-12 and favor the development of a strong cellular immune response. Th2 cells produce IL-4, IL-10, and IL-13 and favor a strong humoral immune response (4, 5). The latter type of immune response appears to be associated with disease progression in RCC. It has been found that higher amounts of IL-4, IL-5, IL-6, and IL-10 are produced in the tumor tissues of patients with higher-stage tumors. In addition, an elevated pretreatment serum level of IL-10 was shown to be a significant independent predictor of poor prognosis in advanced patients (6, 7). Of these Th2 cytokines, IL-4 is intriguing in that high-affinity IL-4R is expressed on a variety of nonhematopoietic tumor cells such as melanoma, ovarian, breast, and renal carcinoma cells (8). IL-4R is a heterodimeric comprising the IL-4Rα and γc chains (9). In fact, signaling through the receptors of IL-4 has also some direct biological effects on these nonhematopoietic tumor cells, such as the production of IL-6 (10, 11). Recently, it was reported that there exist two types of polymorphic sites on the *IL-4R*α gene, and both of them were associated with an increased risk of atopy (12, 13). These polymorphic sites result in the substitution of Ile for Val (*Ile50Val*) and Arg

Received 3/7/02; revised 5/13/02; accepted 5/15/02.
The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
[1] Supported by grants from the Public Trust Haraguchi Memorial Cancer Research Fund and the Shimizu Foundation for the Promotion of Immunology Research, and by a grant from the Ministry of Education, Culture, Sports, Science and Technology, Japan.
[2] E. N. and Y. M. contributed equally to this work.
[3] To whom requests for reprints should be addressed, at Department of Urology, Kyoto University Graduate School of Medicine, Kyoto 606-8507, Japan. Phone: 81-75-751-3325 or 3326; Fax: 81-75-761-3441; E-mail: ogawao@kuhp.kyoto-u.ac.jp.

[4] The abbreviations used are: RCC, renal cell carcinoma; IL, interleukin; IL-4R, IL-4 receptor; OR, odds ratio; CI confidence interval; TNM, tumor-node-metastasis; STAT, signal transducer and activator of transcription.

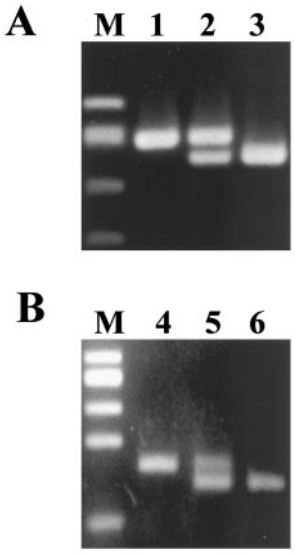

Fig. 1 Genotyping of the polymorphisms of *IL-4Rα* by a mismatch PCR-RFLP method. *A*, *Lane M*, ϕX174-HaeIII marker, *Lane 1*, *Ile/Ile*; *Lane 2*, *Ile/Val*; *Lane 3*, *Val/Val*. *B*, *Lane M*, ϕX174-HaeIII marker, *Lane 4*, *Gln/Gln*; *Lane 5*, *Arg/Gln*; *Lane 6*, *Arg/Arg*.

Table 1 Clinicopathological characteristics of the 143 RCC patients

Variable	No. of patients (%)
All patients	143
Male/Female	108/35
Age, yr	
Median ± SD	60.5 ± 11.4
Range	31–84
Tumor Size (cm)	
Median ± SD	5.4 ± 3.0
Range	1.0–20
Grade[a]	
G1–2	104 (72.7)
G3	33 (23.1)
Undefined	6 (4.2)
TNM stage[b]	
T_1	95 (66.4)
T_2	19 (13.3)
T_3	26 (18.2)
T_4	3 (2.1)
N_+ or M_+	25 (17.5)
N_0M_0	118 (82.5)

[a] Tumor grade according to the 1997 AJCC grading system (15).
[b] Tumor stage according to TNM (14).

for Gln (*Arg576Gln*) in the extracellular and cytoplasmic domain, respectively. Functional assays have shown that substitution of Ile for Val enhances signal transduction via the IL-4R by augmenting the activation of STAT6, whereas the Arg variant strengthened them by impairing the binding of the negative regulatory molecule, protein tyrosine phosphatase SHP-1. Therefore, it is believed that both the *Ile* and *Arg* alleles, which strengthen signals through the IL-4R, were highly distributed in patients with atopy by their genetic effects of predisposing the host immune system to Th2 (12, 13).

To clarify the significance of IL-4 and polymorphisms of the *IL-4Rα* gene in RCC patients, we have examined how this polymorphism influenced the incidence and prognosis in a series of 143 sporadic RCCs in a Japanese population.

MATERIALS AND METHODS

Subjects. The institutional review board of the Kyoto University Graduate School of Medicine approved this study. A total of 348 subjects, consisting of 143 sporadic RCC patients and 205 controls were enrolled after receiving appropriate informed consent. All of the RCC patients were diagnosed histologically with specimens obtained from primary tumors or metastatic lesions, or by autopsy at Kyoto University Hospital or a related community hospital during the 10-year period between 1990 and 1999. Tumors were staged using the TNM system for RCC and graded using the three-grade system (14, 15). Their operations consisted of radical nephrectomy in 115 cases, partial nephrectomy in 22 cases, and metastatectomy in 2 cases. Two patients were treated by *trans*-arterial embolization of primary lesions. After surgery or embolization, patients with metastatic disease, including those who developed recurrence after surgery on the primary lesion, received systemic immunotherapy. IFN-α was administered alone to 21 patients, IFN-γ alone to 4 patients, IFN-α+IFN-γ to 4 patients, IFN-α+5-fluorouracil to 5 patients, and IFN-α+IL-2 to 4 patients (16, 17). Treatment was continued until apparent disease progression. Twenty-five patients died of cause-specific disease and 15 patients were alive with disease. No systemic immunotherapy was given to two patients because of poor performance status.

Genotyping of Two *IL-4Rα* Gene Polymorphisms. Genomic DNA was obtained from a blood sample or the normal kidney of each patient, as described previously (3). The *IL-4Rα* gene polymorphisms were examined by a mismatch PCR-RFLP method using the primers and enzymes described below. All of the DNA samples were analyzed twice, and samples homozygous for the restriction site were used as a positive control in each digestion to avoid false results by incomplete enzyme digestion.

***Ile50Val* (Ile for Val Substitution).** The *Ile50Val* polymorphism was genotyped by a mismatch PCR-RFLP method. The 273-bp fragment in which the *Rsa*I restriction site was introduced by a single-basepair mismatched antisense primer was amplified using primers and PCR conditions described previously by Noguchi *et al.* (18). PCR products were digested with *Rsa*I and electrophoresed in 1.5% agarose + 3.0% NuSieve agarose gel (FMC BioProducts, Rockland, ME; Fig. 1*A*).

***Arg576Gln* (Arg for Gln Substitution).** The *Arg576Gln* polymorphism was genotyped by a mismatch PCR-RFLP method described previously by Noguchi *et al.* (19). Briefly, a single basepair mismatch was introduced in the sense primer to obtain the *Msp*I restriction site, and digested PCR fragments were analyzed using agarose gel electrophoresis (Fig. 1*B*).

Statistical Analysis. We used the χ^2 test to evaluate whether the distribution of genotypes varied significantly between RCC and controls. The linkage disequilibrium between the two polymorphisms was evaluated by a χ^2 test. Cause-specific survival was defined as the time from surgery to death if the patient died from RCC, or to last contact. Survival curves

Table 2 The distribution of polymorphisms in the *IL-4Rα* gene in RCC patients and controls

Origin	Genotype					Genotype				
	Ile/Ile	*Ile/Val*	*Val/Val*	OR (95% CI)[a]	*P*	*Arg/Arg*	*Arg/Gln*	*Gln/Gln*	OR (95% CI)[b]	*P*
References[c]	49 (15.3)	142 (44.2)	130 (40.5)			6 (2.2)	51 (18.4)	221 (79.8)		
Control (M = 122, F = 83)	27 (13.1)	94 (45.9)	84 (41.0)			2 (1.0)	42 (20.5)	161 (78.5)		
RCC (M = 110, F = 33)	25 (17.5)	76 (53.1)	42 (29.4)	2.0 (1.38–2.90)	0.026	5 (3.5)	40 (30.0)	98 (68.5)	1.68 (1.03–2.73)	0.036

[a] *Ile/Ile + Ile/Val versus Val/Val.*
[b] *Arg/Arg + Arg/Gln versus Gln/Gln.*
[c] Values are taken from previous studies of Japanese populations (13, 18, 19, 24, 25).

were generated by the Kaplan-Meier method, and differences between groups defined by genotypes of the *IL-4Rα* gene were compared by the log-rank test. To produce multivariant models of cause-specific survival, variables including clinicopathological parameters, previously described as predictors of prognosis of RCC (20) and revealed to be significant by univariate analysis including genotypes of *IL-4Rα*, were assessed for relative risk, 95% CI, and *P*s using the Cox proportional hazards model (21). Clinicopathological parameters and genotypes of the *IL-4Rα* gene were dichotomized as follows: T stage (T_{1-2} *versus* T_{3-4}), nodal status (>1 *versus* no positive lymph nodes), tumor grade (1–2 *versus* 3), genotypes of the *IL-4Rα* gene (*Ile/Ile versus* others, *Gln/Gln versus* others). All of the statistical analyses were performed using statistical software (StatView version 5.0, SAS Institute, Inc. NC). Statistical significance in this study was set at $P < 0.05$. All of the reported *P*s are two-sided.

Table 3 Linkage disequilibrium between the *Ile50Val* and *Arg576Gln* polymorphisms in controls[a]

	Arg576Gln			
	Arg/Arg	*Arg/Gln*	*Gln/Gln*	Total
Ile50Val				
Ile/Ile	1	7	19	27
Ile/Val	1	28	65	94
Val/Val	0	7	77	84
Total	2	42	161	205
Agreement			0.61	
Expected agreement			0.527[b]	
P			<0.01	

[a] *P* is calculated with χ^2 test between agreement and expected agreement.
[b] Expected agreement under the assumption of no linkage disequilibrium.

RESULTS

Patients and Clinicopathological Parameters. Table 1 shows the clinicopathological characteristics of the 143 sporadic RCC patients. The mean age of the patients at initial presentation was 60.5 years (range, 31–84). A total of 25 patients showed nodal involvement or distant metastasis at initial presentation. The distribution of the clinical findings was in good agreement with previously reported data of RCC in Japanese populations (22, 23), which indicated that the present population was representative of RCC patients in the Japanese population.

Allele Frequencies. Table 2 shows the distribution of both alleles in the references from previous studies (13, 18, 19, 24, 25), present controls, and the 143 sporadic RCC patients. The allele frequencies in control individuals were consistent with those of previous studies. Similar to the atopic population, the frequencies of both *Ile* and *Arg* alleles among RCC patients were significantly higher than controls with OR of 2.0 [(*Ile/Ile+Ile/Val versus Val/Val*), 95% CI, 1.38–2.90; $P = 0.026$] and 1.68 [(*Arg/Arg+Arg/Gln versus Gln/Gln*), 95% CI, 1.03–2.73; $P = 0.036$]. Thus, these findings indicate that alleles associated with enhanced signaling of the IL-4 were correlated with increasing risks of RCC. We then further examined whether the linkage disequilibrium was present between the two polymorphisms by analyzing their distribution in the controls (Table 3). Assuming that *Ile* and *Val* alleles in *Ile50Val* are in disequilibrium with *Arg* and *Gln* alleles in *Arg576Gln*, respectively (*i.e.*, an excess of *Ile/Arg* and *Val/Gln* haplotypes), the observed agreement was 61.0%. This was significantly higher than the expected agreement (52.7%; $P < 0.01$), which indicated that linkage disequilibrium existed between these polymorphic sites. Thus, it remains unclear whether both or one of the alleles are responsible for the genetic effects on the incidence of RCC.

Association of Polymorphisms of the *IL-4Rα* Gene with Clinicopathological Stage and Patients Survival. Next, we examined their association with clinicopathological stage. No significant association was observed between the *Arg576Gln* polymorphism and the clinicopathological stage at initial presentation (Table 4). However, patients with the *Ile/Ile* genotype showed a trend toward exhibiting higher tumor stages than the *Ile/Val* or *Val/Val*. As many as 32% (8 of 25) of patients had distant or lymph-node metastasis in the Ile homozygotes, whereas 14.4% (17 of 118) of patients in other genotypes did so. These findings suggest that polymorphisms of the *IL-4Rα* gene may influence disease progression and prognosis of RCC patients. To clarify this, cause-specific survival curves were generated by the Kaplan-Meier method. Differences in survival between genotypes of the *IL-4Rα* gene were compared by the log-rank test. As expected, patients with the *Ile/Ile* genotype had a significantly lower cause-specific survival compared with those with other genotypes (*Ile/Ile versus Ile/Val*: $P = 0.0011$; *Ile/Ile versus Val/Val*: $P = 0.015$; Fig. 2*A*). These findings indicate that the *Ile* allele has a recessive genetic effect on the poor prognosis of RCC patients and is similar to that of the atopic population in that the genetic effect turned out to be most apparent in homozygotes (13). As for the *Arg576Gln* polymor-

Table 4 Relationship between the *IL-4Rα* gene polymorphisms and clinicopathological stage of patients

	IL-4Rα genotype					
	Ile/Ile	*Ile/Val*	*Val/Val*	*Arg/Arg*	*Arg/Gln*	*Gln/Gln*
No. of patients	25	76	42	5	40	98
Age at onset, yr	60.7 ± 12.9	61.6 ± 11.3	58.3 ± 11.2	54.6 ± 13.4	62.8 ± 11.8	59.8 ± 11.3
Grade						
G1–2	17	56	31	5	28	71
G3	8	16	9	0	11	22
TNM stage						
T_1	15 (60.0)	48 (63.2)	29 (69.0)	4 (80)	21 (52.5)	67 (68.4)
T_2	4 (16.0)	9 (11.8)	5 (11.9)	0 (0)	9 (22.5)	9 (9.2)
T_3	5 (20.0)	14 (18.4)	7 (16.7)	1 (20)	6 (15)	19 (19.4)
T_4	1 (4.0)	1 (1.3)	1 (2.4)	0 (0)	0 (0)	3 (3.1)
M_+ or N_+	8 (32.0)	11 (14.5)	6 (14.3)	0 (0)	8 (20)	17 (17.3)
M_0N_0	17 (68.0)	65 (85.5)	36 (85.7)	5 (100)	32 (80)	81 (82.7)

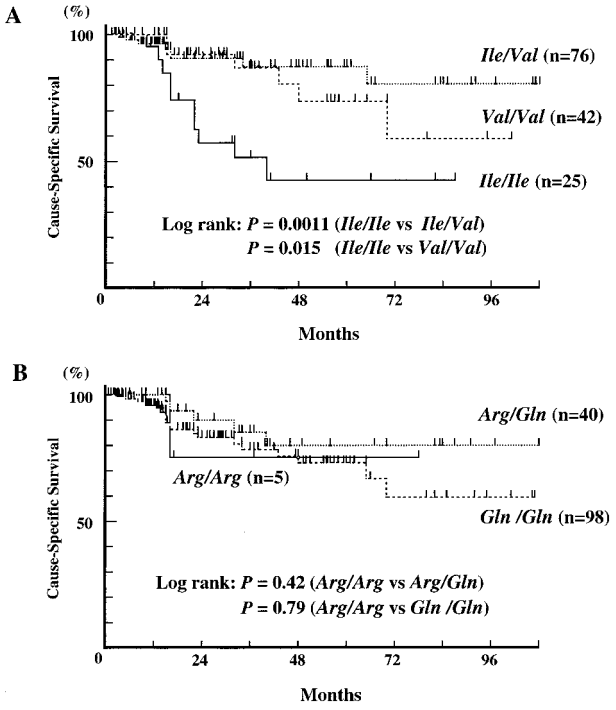

Fig. 2 Cause-specific survival for 143 patients with RCC according to the genotype of the *IL-4Rα* gene. *A*, a significant difference in survival was obtained between groups categorized by the *Ile50Val* genotype. *B*, the *Arg576Gln* genotype did not significantly influence cause-specific survival of patients. Cause-specific survival was analyzed using the Kaplan-Meier method, and comparison of groups was performed with the log-rank test.

phism, no association was observed between genotype and prognosis of RCC patients (*Arg/Arg versus Arg/Gln*: $P = 0.42$; *Arg/Arg versus Gln/Gln*: $P = 0.79$; Fig. 2*B*).

Prognostic Significance of Polymorphisms of the *IL-4Rα* Gene. To further examine the correlation between polymorphisms of the *IL-4Rα* gene and the prognosis of RCC patients, the clinicopathological stage and genotypes of the *IL-4Rα* gene were examined by a multivariate regression analysis using a Cox proportional hazard model. Univariate analyses

Table 5 Univariate and multivariate analysis of clinicopathological stage and the *IL-4Rα* gene polymorphisms for cause-specific survival

		Multivariate		
Factors	Univariate *P*	RR[a]	95% CI	*P*
TNM stage				
T	<0.0001	1.3	0.48–3.5	0.61
N	<0.0001	1.8	0.51–6.4	0.37
M	<0.0001	6.8	2.5–18.9	0.0002
Grade				
G1–2 *vs.* G3	<0.0001	2.8	1.0–31.8	0.044
IL-4Rα				
Ile50Val[b]	0.0014	3.4	1.2–9.3	0.018
Arg576Gln[c]	0.27			

[a] RR, relative risk.
[b] *Ile/Ile versus Ile/Val + Val/Val.*
[c] *Arg/Arg + Arg/Gln versus Gln/Gln.*

using the log-rank test identified tumor (T) classification ($P < 0.0001$), *n* classification ($P < 0.0001$), metastasis (M) classification ($P < 0.0001$), histopathological grade ($P < 0.0001$), and Ile variants of the *IL-4Rα* gene (*Ile/Ile versus Ile/Val+Val/Val*: $P = 0.0014$) as significant prognostic predictors for cause-specific survival. Then, multivariate analyses were performed for polymorphisms of the *IL-4Rα* gene together with independent prognostic variables with univariate statistical significance. Homozygosity of the *Ile* allele of the *IL-4Rα* gene was an independent prognostic factor for cause-specific survival (*Ile/Ile versus Ile/Val+Val/Val*: relative risk = 3.4; $P = 0.018$). Metastasis classification and histopathological grade also had prognostic value with a relative risk of 6.8 ($P = 0.0002$) and 2.8 ($P = 0.044$), respectively (Table 5). These findings were compatible with those of a previous study of RCC in a Japanese population examined by multivariate analyses (23).

DISCUSSION

In this study, we show that genetic polymorphisms of the *IL-4Rα* gene are associated with the incidence and prognosis of sporadic RCC in a Japanese population. In comparison to healthy controls, it was shown that both *Arg* and *Ile* alleles, which in lymphoid cells enhance signaling through the IL-4R (9, 12, 13), had dominant genetic effects on increasing risks of

sporadic RCC. Because this receptor is also expressed on freshly isolated RCC cells (10, 26), it is possible that signals through the receptor are responsible for this genetic effect. At present, the effect of IL-4 and signals through IL-4R for RCC cells still remains controversial. It has been shown in primary cultures of RCC tumor cells that IL-4 had inhibitory effects for the growth of the tumor cells in a dose-dependent manner (10). However, in response to IL-4, the RCC cell line Caki-1 produces IL-6, one of the representative growth factors that lead renal tumor cells to further transformation and proliferation (11, 27). Although, the exact roles of IL-4 and its signaling pathway on RCC are still unknown and require further examination, it is possible that the dominant genetic effects of the polymorphisms on increasing risks of RCC are attributable to the direct effects of signals through the IL-4R. In addition, patients homozygous for the *Ile* allele exhibited unfavorable prognosis compared with those with other genotypes (Fig. 2). Moreover, this genotype was identified as an independent prognostic factor for cause-specific survival by multivariate analyses using representative prognostic variables (Table 5). Because patients carrying the same genotype are at higher risk of atopy by the predisposition of their immune system to Th2 (13, 25), this may also cause the unfavorable prognosis of these patients. This assumption is further supported by some important clinical aspects of RCC patients in that higher amounts of Th2 cytokines are produced in advanced-stage disease of patients, and the serum level of IL-10 was identified as a significant independent prognostic factor for patients (6, 7). In fact, it is also the case with other kinds of malignancies in that the polarization of the host immune system to Th2 is associated with the disease progression (28–30). Although it is suggested from the murine tumor model that Th1-dominant immunity plays a critical role in the induction of antitumor immunity *in vivo*, the precise mechanisms are still undefined (31). Further clarifications of roles of the Th1/2 immune system in antitumor immunity are required for the improvement of the prognosis of not only the RCC but also other types of cancer patients.

One of the unresolved issues raised by the present study is whether or not both polymorphic sites of the *IL-4Rα* gene are associated with the increasing risks of RCC. Although the presence of the linkage disequilibrium should be confirmed by haplotype analyses with a greater number of subjects, our present results suggested the possibility that the linkage disequilibrium between the two markers caused the positive association of both polymorphisms with those risks. To resolve this, further examination is required, *e.g.*, how the polymorphisms affect the production of IL-6 or cell proliferation in RCC cell lines. However, the effect of the *Arg576Gln* variant in the signal transduction pathways via IL-4R was not replicated in several recent functional assays (13, 25, 32, 33). So, it is probable that the *Arg* allele might appear to exhibit its association with increasing risks of RCC merely in linkage with the *Ile* allele.

Because various environmental factors such as cigarette smoking, obesity, and hypertension are known to increase the risk of RCC (1, 2), it remains possible that genetic variants of the *IL-4Rα* gene are merely in linkage with another important RCC-susceptible gene. In addition, because the IL-4Rα chain is also an essential component of the receptor for IL-13 (34), another representative Th2 cytokine, we cannot exclude the possibility that the effects of these polymorphisms are also caused by signaling through that receptor. However, the present preliminary findings suggest that polymorphisms of the *IL-4Rα* gene may serve as useful genetic markers for assessing the increasing risks of RCC and also the probability that the polarization of the host immune system to Th2 causes the unfavorable prognosis of patients with RCC. Additional studies in other and larger populations are necessary to confirm the present observation, and it is also necessary to examine the influences of these polymorphisms on the Th1/Th2 profiles of cytokines produced locally in the tumors or in the serum of RCC patients.

ACKNOWLEDGMENTS

We thank Drs. William Kim and Chikuma Hamada for useful suggestions and comments on the manuscripts.

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Clin Cancer Res 2002;8:2620-2625.