VOLUME 25 · NUMBER 7 · MARCH 1 2007

JOURNAL OF CLINICAL ONCOLOGY

ORIGINAL REPORT

Interleukin-4 Promoter Polymorphisms: A Genetic Prognostic Factor for Survival in Metastatic Renal Cell Carcinoma

Thomas Kleinrath, Christoph Gassner, Peter Lackner, Martin Thurnher, and Reinhold Ramoner

From the Central Institute for Blood Transfusion and Immunological Department, Department of Neurology, and Department of Urology, Innsbruck Medical University, Innsbruck, Austria.

Submitted June 9, 2006; accepted December 6, 2006.

Supported by Grant No. 03a of the Kompetenzzentrum Medizin Tirol (to M.T., work was performed in the Department of Urology).

All institutions are certified according to International Standards Organization 9001:2000.

Both T.K. and C.G. contributed equally to this work.

Authors' disclosures of potential conflicts of interest and author contributions are found at the end of this article.

Address reprint requests to Reinhold Ramoner, PhD, Department of Urology, Innsbruck Medical University, Anichstr 35, Innsbruck, Ty, Austria; e-mail: reinhold.ramoner@i-med.ac.at; *cc*: martin.thurnher@i-med.ac.at.

0732-183X/07/2507-845/$20.00

DOI: 10.1200/JCO.2006.07.8154

ABSTRACT

Purpose
Renal cell carcinoma (RCC) is considered a cytokine-responsive tumor. The clinical course of a patient may thus be influenced by the patient's capacity to produce distinct cytokines. Therefore, cytokine gene polymorphisms in RCC patients were analyzed to determine haplotype combinations with prognostic significance.

Patients and Methods
A selection of 21 single nucleotide polymorphisms within the promoter regions of 13 cytokine genes were analyzed in a cross-sectional single-center study of 80 metastatic RCC patients. Univariate and multivariate analyses and the Cox forward-stepwise regression model were chosen to assess genetic risk factors.

Results
Multivariate Cox regression analysis confirmed by a bootstrap technique identified the heterozygous *IL4* genotype −589T−33T/−589C−33C as an independent prognostic risk factor (risk ratio, 3.1; $P < .01$; 95% CI, 1.4 to 6.9; adjusted for age, sex, and nuclear grading) in metastatic RCC patients. *IL4* haplotype −589T−33T and −589C−33C were found with a frequency of 0.069 and 0.925, respectively, which represents a two-fold decrease of *IL4* haplotype −589T−33T ($P < .01$) and an increase of *IL4* haplotype −589C−33C frequency ($P < .05$) in metastatic RCC compared with other white reference study populations. The median overall survival was decreased 3.5-fold ($P < .05$) in heterozygote patients carrying *IL4* haplotype −589T−33T and −589C−33C (3.78 months) compared with patients homozygote for *IL4* haplotype −589C−33C (13.44 months). In addition, a linkage disequilibrium between the *IL4* gene and the *KIF3A* gene was detected.

Conclusion
Our findings indicate that *IL4* promoter variants influence prognosis in patients with metastatic RCC and suggest that genetically determined interleukin-4 (IL-4) production affects the clinical course of the disease possibly through regulation of immune surveillance.

J Clin Oncol 25:845-851. © 2007 by American Society of Clinical Oncology

INTRODUCTION

Renal cell carcinoma (RCC) is resistant to conventional therapies such as radiation, hormone therapy, and chemotherapy. At diagnosis, 20% to 30% of patients with RCC already have metastatic disease and 20% to 40% of patients with organ-confined tumors at nephrectomy will develop metastatic disease.[1] Metastatic disease is characterized by a poor prognosis, with a median survival of 13 months.[2] RCC tissue frequently is infiltrated by lymphocytes,[3] macrophages,[4] and dendritic cells,[5] reflecting the immunogenicity of RCC.

RCC has also been shown in vitro to respond to various cytokines including tumor necrosis factor alpha (TNF-α),[6] interleukin-6 (IL-6),[7] type 1 interferon (IFN),[8] IFN gamma (IFN-γ),[9] IL-4,[10-13] and IL-13.[14] The cytokine effects observed in these studies were either growth-promoting (TNF-α, IL-6) or antiproliferative (type 1 IFN, IFN-γ, IL-4, and IL-13). For IL-4 and IL-13, growth-inhibitory effects on RCC in vitro via binding of the cytokines to their specific receptors have been reported.[11,14] However, despite these pronounced preclinical observations, subsequent clinical trials of systemic IL-4 in patients with RCC and other tumors did not indicate significant beneficial effects.[15-17] In contrast, treatment with type 1 IFN and IL-2 showed a modest survival benefit.[2,18]

Cytokines and their receptors are known to be highly conserved within their coding region.[19,20] However, several examples of amino acid sequence

variations have been described in healthy individuals.[21] Although polymorphisms outside of the coding region do not influence the amino acid sequence, they may affect protein expression by influencing alternative mRNA splicing, mRNA stability, or transcription levels.[22,23] Especially polymorphisms within the 5′- and 3′-regulatory regions of cytokines are of crucial impact, given that they can determine transcription factor binding sites within the cytokine gene promoters as well as the structure of enhancers and silencers.[21] In part, human diversity in immune responses may be explained by such polymorphisms because they lead to interindividual differences in cytokine-producing capacity, which results in a variety of biologic consequences.

For example, findings of different studies showed that the *IL4* promoter variants *IL4*/−589T and *IL4*/−33T lead up to a three-fold higher transcriptional activity in vitro, which was subsequently confirmed in vivo.[24-26] *IL4*/−589, *IL4*/−33, and other noncoding variations of *IL4* were also shown to influence the risk of Th2-mediated diseases such as asthma and atopy, and to be involved in the regulation of serum immunoglobulin E levels, which is one of the major clinical characteristics of these diseases.[25,27,28]

To elucidate further a potential role of endogenous cytokines in the course of metastatic RCC, a representative selection of interindividual cytokine and cytokine receptor gene promoter polymorphisms were investigated in 80 RCC patients.

PATIENTS AND METHODS

Study Population

Eighty-nine white patients were selected in a single-center study. Nine patients were excluded from analysis because of low DNA quality or poor DNA yield. All patients had RCC with clear-cell histology and had already developed bidimensionally measurable metastatic lesions. Furthermore, all patients were participants in the local dendritic cell vaccine program (1998 to the end of 2004).[29,30] The primary tumor was removed in all patients and the start of follow-up was defined by the beginning of immunotherapy. Patients with solitary brain metastasis, other malignancies than RCC within the last 5 years, treatment with immunosuppressive drugs, other immunotherapies or chemotherapies within 4 weeks before treatment start, pregnancy or lactation, presence of acute or chronic infections, HIV or viral hepatitis, or a Karnofsky performance score less than 60 were excluded from the study. Furthermore, a computed tomography of brain, chest, and abdomen and a bone scan were performed. All patients were informed about the investigative character of the study and provided their written informed consent.

Cytokine Genotyping and DNA Preparation

Genomic DNA was isolated from frozen peripheral blood mononuclear cells using Nucleon BACC2 reagents (Amersham, Buckinghamshire, United Kingdom).

A selection of 21 single nucleotide polymorphisms (SNPs) within 13 genes (*IFNG*, *IL1A*, *IL1B*, *IL1R1*, *IL1RN*, *IL2*, *IL4*, *IL4R*, *IL6*, *IL10*, *IL12B*, *TGFB1*, and *TNF*) was typed using the Cytokine CTS-PCR-SSP Tray Kit as described by the manufacturer (Department of Transplantation Immunology, University of Heidelberg, Heidelberg, Germany) according to procedures recommended by the 13th International Histocompatibility Workshop Cytokine Polymorphisms Component.[31]

Details on specificities, primer sequences, and reaction conditions of the Cytokine CTS-PCR-SSP Tray Kit are available for *IL10*, *TGFB1*, *TNF*, *IL4R*, *IL1B*, *IL1RN*, *IL1R1*, *IL1A*, *IL4*, and *IL6*.[32-34] Typing results for SNP rs2243248 did not meet quality requirements for interpretation and were therefore excluded from additional analysis.

Many different aliases for the same polymorphism are found in the literature. Some authors refer to the position relative to the transcriptional start site, whereas others use the position relative to the "A" of the start codon ATG,

Table 1. SNPs Detected by Heidelberg Cytokine Genotyping Kit

Gene	Reaction No. in Genotyping Kit	dbSNP-ID	Allele 1	Allele 2	SNP Position*	Trivial Name in Heidelberg Kit	SNP Aliases, Commonly Used in Literature	MAF	Source of MAF†	Reference
IL1A	1-2	rs1800587	C	T	−1613	−889	−889	0.308	A	39
IL1B	3-4	rs16944	C	T	−1058	−511	−511	0.345	A	40
	5-6	rs1143634	C	T	3407	3962	3954	0.223	A	41
IL1R1	7-8	rs2234650	C	T	−15858	pst1 1970	pst I A	NA	NA	37
IL1RN	9-10	rs315952	T	C	5096	mspa1 11,100	pos 30,735	0.258	A	42
IL2	27-30	rs2069762	T	G	−385	−330	−714	0.232	A	35
		rs2069763	G	T	114	166	114	0.242	B	43
IL4	31-38	rs2243248‡	T	G	−1099	−1098	NA	0.082	A	NA
		rs2243250	C	T	−589	−590	C-589T,−524	0.100	B	27,44
		rs2070874	C	T	−33	−33	C-33T	0.158	A	27
IL4R	11-12	rs1801275	A	G	22883	1902	Gln551Arg	0.225	A	45
IL6	39-42	rs1800795	G	C	−237	−174	−174	0.467	A	46
		rs1800797	G	A	−661	nt565	−597	0.481	A	47
IL10	43-48	rs1800872	C	A	−627	−592	−592	0.185	A	48
		rs1800871	C	T	−854	−819	−819	0.173	A	49
		rs1800896	A	G	−1117	−1082	−1082	0.458	A	48
IL12B	13-14	rs3212227	C	A	10842	−1188	pos 1188	0.225	A	50
IFNG	15-16	rs2430561	A	T	874	874	874	NA	NA	51
TGFB1	17-22	rs1982073	T	C	29	Codon 10	869	0.451	B	35
		rs1800471	G	C	74	Codon 25	915	0.113	B	52
TNF	23-26	rs361525	G	A	−419	−238	−238	0.033	B	53
		rs1800629	G	A	−489	−308	−308	0.167	B	54

Abbreviations: db, database; SNP, single nucleotide polymorphism; MAF, minor allele frequency; rs, reference SNP; NA, not available; NCBI, National Center for Biotechnology Information.
*Position relative to ATG, including SNP nucleotide (positive or negative).
†Sources for MAF data were from HapMap project[55] or NCBI (www.ncbi.nlm.nih.gov) for the white CEU- and CAUC1 panels, respectively.
‡Typing results for SNP rs2243248 did not meet quality requirements for interpretation and were therefore excluded from additional analysis.

Table 2. Patient Characteristics

Characteristic	No. of Patients	%
Median age, years	56	
Sex		
Female	29	36
Male	51	64
Median follow-up, months	20	
Site of metastasis		
Adrenal gland		
Yes	7	9
No	73	91
Bone		
Yes	19	24
No	61	76
Brain		
Yes	7	9
No	73	91
Liver		
Yes	16	20
No	64	80
Lung		
Yes	61	76
No	19	24
Lymph node		
Yes	27	34
No	53	66
Skin		
Yes	5	6
No	75	94
Prior therapy		
Cytokine (interferon alfa, interleukin-2, or both)	8	10
Chemotherapy (cytotoxic)	4	5
Chemotherapy and cytokine therapy	6	8
Median baseline laboratory parameters (n = 44)		
Hemoglobin, g/L	12.6	
Lactate dehydrogenase, U/mL	180.6	
Total calcium level, mmol/L	2.38	

the position according to the GenBank-sequence used, or simply refer to a restriction enzyme used.[27,35-37]

We followed the recommendations of the Human Genome Variation Society[38] by giving detailed data on all detectable SNPs of the Cytokine CTS-PCR-SSP Tray Kit including the corresponding database SNP (dbSNP) identifications (reference SNP [rs] numbers), the positions relative to the "A" of the start codon ATG, and commonly used SNP aliases (Table 1).[27,35,37,39-55]

Table 3. Multivariate Analysis

Factor	SE	Risk Ratio	*P*	95% CI
Age	0.01	1.0	.953	1.0 to 1.0
Sex	0.26	1.3	.386	0.7 to 2.3
Nuclear grade				
I and II	0.33	1.0 (reference)	<.001	
III	0.41	2.0	<.05	1.0 to 3.7
IV	0.01	5.4	<.001	2.4 to 11.9
IL4 genotypes	0.41	3.1	<.01	1.4 to 6.9

Eighty patients were typed for all SNPs described above. For six of these genes (*IL2*, *IL4*, *IL6*, *IL10*, *TGFB1*, and *TNF*), a typing method also known as double amplification refractory mutation system–polymerase chain reaction[56] was chosen, which allowed the detection of haplotypes instead of simply the analysis of SNPs. Hence, for each of the above-mentioned genes, four haplotypes could be defined.

Linkage Disequilibrium Analysis

For linkage disequilibrium (LD) analysis, SNP genotype data (CEU [CEPH: Utah residence with ancestry from Northern and Western Europe] population) of a 140-kbp region—containing the cytokine genes *IL13* and *IL4*, as well as RAD50 homolog (*RAD50*) and kinesin family member 3A (*KIF3A*)—were exported from the HapMap project database[55] and analyzed using the Haploview software version 3.32 (www.broad.mit.edu/mpg/haploview/). An LD map was created using the "Solid spine of LD" setting of the Haploview software.

Statistical Analysis

To identify genotyping errors, the Hardy-Weinberg equilibrium was calculated within population-based data sets.[57] The false-discovery rate criterion, which has been proposed as an appropriate statistical method in genetics research, was used for controlling the errors in multiple comparisons.[58] The Hardy-Weinberg equilibria for all polymorphisms (n = 13) were in the normal range (corrected $P > .3$). Subsequently, multivariate analysis was done using the Cox forward-stepwise regression model. In the stepwise procedure, a significance level of .05 for entering and .10 for removing the respective explanatory variables was used to determine the independent risk factors. A bootstrap technique[59] was applied to confirm the choice of variables. One thousand bootstrap samples were created and stepwise Cox regression was applied to each sample. The percentage of samples for which each variable was included in the model from the 1,000 samples was calculated. After having identified the *IL4* promoter polymorphisms to be the best independent risk factor predicting survival, gene allele frequency comparison was calculated using χ^2 test (two-tailed, significance level = .05). The *IL4* heterozygote genotype 2/4 was observed in only one of the 80 patients and was therefore excluded from the survival analysis. The Kaplan-Meier method and log-rank test were used to assess the prognostic significance of the respective cytokine genotypes for overall survival.

RESULTS

This study was conducted during a 6-year period in 80 patients with metastatic RCC. All patients had clear cell histology. Median age of patients was 56 years at the beginning of the study, with a median overall survival of 12.6 months. Sixty-one patients (76%) already had lung metastases and seven patients (9%) developed brain metastases during follow-up (Table 2). The median follow-up was 19.7 months. Sixty-four percent of the study population was male and 36% was female. All patients were enrolled for dendritic cell vaccination protocols.[29,30]

Multivariate Analysis

First, a multivariate analysis of the effects of genotype on survival was conducted using Cox proportional hazards models to adjust for confounding factors. Using a significance level of .05 for entering and .10 for removing a variable in a stepwise cox regression, four variables were selected: tumor grading, *IL4, IL10*, and transforming growth factor beta (*TGFβ1*). However, when fitting the final model with these four variables, as well as sex and age as independent predictors, *TGFβ1* and *IL10* showed a *P* value of more than .05. Therefore, *TGFβ1* and *IL10* were not included in the final model. To confirm this decision, a bootstrapping technique was applied. The percentage of inclusion among the 1,000 samples created by the bootstrapping technique for

Table 4. Comparison of *IL4* Haplotype Frequencies and Corresponding IL-4 Expression As Published Previously

IL4 Haplotype Identification	rs2243250 −589*	rs2070874 −33*	IL-4 Expression in T Cells[24-26]	Previously Published[60] No. (n = 1,310)	Previously Published[60] Frequency	Previously Published[60] *P*	Locally Matched Healthy Control† No. (n = 446)	Locally Matched Healthy Control† Frequency	Locally Matched Healthy Control† *P*	Renal Cell Carcinoma Patients No. (n = 160)	Renal Cell Carcinoma Patients Frequency
1	T	T	High	189	0.144	0.009	48	0.108	.155	11	0.069
2	T	C	High	5	0.004	0.649	4	0.009	.744	1	0.006
3	C	T	Low	0	0.000	ND	3	0.007	ND	0	0.000
4	C	C	Low	1,116	0.852	0.012	391	0.877	.095	148	0.925

NOTE. Haplotype frequencies between renal cell carcinoma patients and the previously published cohort or the locally matched healthy control group respectively were compared using the χ^2 test.
Abbreviations: rs, reference SNP; ND, not determined; SNP, single nucleotide polymorphism.
*SNP position relative to ATG, including SNP nucleotide (positive or negative; see also Table 1).
†Unpublished observation, C.G., March 2005.

tumor grading and *IL4* were 99.9% and 93.4%, respectively. In contrast, the percentage of inclusion for *TGFβ1* and *IL10* was lower than 60%. The results of the bootstrap procedure confirmed the variables chosen for the final model. Among these 13 promoter variations, the *IL4* promoter polymorphisms −589/−33 turned out to be the best independent risk factor predicting survival. The *IL4* genotype 1,4 was found in 11 patients, showing a 3.1-fold increased risk ratio ($P < .01$) adjusted for age, sex, and grading (Table 3).

Frequencies of IL4 Haplotypes 1 and 4 in Metastatic RCC

In white control populations, which include a locally matched control group, *IL4* haplotypes 1 and 4 were found with frequencies ranging from 0.108 to 0.144 and 0.877 to 0.852, respectively.[60,61] In our RCC patient cohort, the frequency of *IL4* haplotype 1 was decreased significantly (0.069; $P < .01$), and at the same time, *IL4* haplotype 4 was increased significantly (0.925; $P < .05$). In line with the study by Beghé et al,[60] a trend for a decreased *IL4* haplotype 4 frequency was also found in the locally matched control population analyzed with the same cytokine genotyping kit for *IL4* haplotype 4 ($P = .095$). Data are summarized in Table 4.[24-26,60]

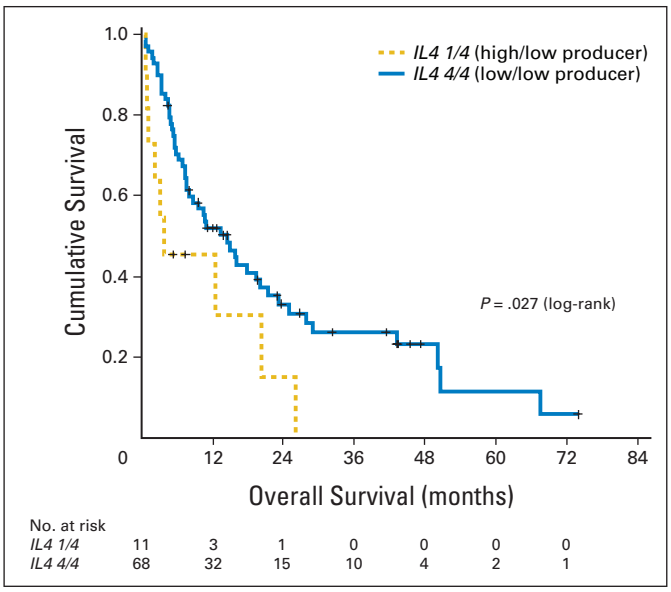

Fig 1. Kaplan-Meier plot of overall survival in relation to the *IL4* genotype.

Univariate Survival Analysis

In the next step, the influence of *IL4* haplotypes on patient survival was examined. Using the Kaplan-Meier method and log-rank test, the only significant association with survival was found for the *IL4* promotor polymorphism. Median overall survival was 3.5-fold increased ($P = .027$) in patients homozygote for *IL4* haplotype 4 (13.44 months) compared with heterozygote patients carrying *IL4* haplotypes 1 and 4 (3.78 months; Fig 1 and Table 5). Thus, patients expressing the *IL4* genotype 1,4 showed a significantly decreased survival. Given the haplotype frequencies observed, the rare *IL4* genotype 1,1 was not encountered in our study group (Table 5).

IL4 haplotypes 1 and 2 both carrying the *IL4*/−589T allele represent two high *IL4*-expressing haplotypes, whereas low-expressing haplotypes 3 and 4 exhibit an *IL4*/−589C allele.[24-26] Hence, the predicted functional phenotypes for the homozygous genotype *IL4* 4,4, the heterozygous genotype *IL4* 1,4, and the homozygous genotype *IL4* 1,1 are low (low/low), intermediate (high/low), and high (high/high) IL-4 producers, respectively (Tables 4 and 5).

Thus, patients with an expected low IL-4–producing phenotype have a 9.7 median months survival benefit compared with patients with an intermediate IL-4–producing phenotype (Fig 1).

LD Analysis of the IL4 region

Finally, we performed an analysis of the LD map for the *IL4* region, containing the genes *RAD50*, *IL13*, *IL4*, and *KIF3A*,

Table 5. Observed *IL4* Genotypes, Frequencies, and Corresponding Median Overall Survival in Renal Cell Carcinoma Patients

IL4 Genotypes*	Observed Frequencies No. (n = 80)	Observed Frequencies Frequency	Median Survival in Renal Cell Carcinoma Patients (months)†	Predicted IL-4 Expression in T Cells[24-26]
1,4	11	0.138	3.78	High/low
2,4	1	0.013	ND	High/low
4,4	68	0.850	13.44	Low/low

Abbreviation: ND, not determined.
*Haplotypes are described in Table 4.
†Figure 1.

which were found in four major linkage blocks. *RAD50* was located in the first, *IL13* in the second, and *IL4* and *KIF3A* in the third −58-kbp block, respectively. No gene was positioned in block four. All *IL4*-specific SNPs analyzed in this study, as well as most of the variations within the complete coding regions of the *IL4* gene and the *KIF3A* gene, showed strong LDs within block three.

DISCUSSION

In this study, we demonstrate for the first time the prognostic role of *IL4* promoter polymorphisms in patients with metastatic RCC. Median overall survival was 3.5-fold decreased in heterozygote patients carrying haplotypes 1 and 4 compared with patients homozygote for haplotype 4 (3.78 *v* 13.44 months; $P = .027$; Fig 1). The heterozygous *IL4* genotype 1,4 (intermediate IL-4 producer) is thus associated with an approximately three-fold increased risk of an unfavorable clinical course of RCC compared with genotype 4,4 (low IL-4 producer). Given that the rare *IL4* genotype 1,1 (high IL-4 producer) was not encountered in our study group, no actual data on the outcome for these patients can be presented. However, it seems plausible that survival of such patients might even be shorter, compared with *IL4* genotype 1,4 heterozygotes (intermediate IL-4 producers).

Another finding of this study was the reduced frequency of the *IL4* haplotype 1, which is associated with decreased survival, and the concomitantly increased frequency of the *IL4* haplotype 4 in metastatic RCC patients compared with other white study populations (Table 4). One possible interpretation of this finding is that rapid disease progression in patients with the *IL4* haplotype 1 may prevent patient enrollment into study protocols due to insufficient performance status, and thus results in a relative paucity of *IL4* haplotype 1 in RCC study populations. However, the issue is complicated further by the observation that the frequency of *IL4* haplotype 1 is increased 5.4-fold in Asians (0.789)[61] compared with whites (0.144).[60] Intriguingly, the substantially increased frequency of the *IL4* haplotype 1 in Asians is associated with a lower incidence of kidney cancer.[62] These data, which may appear contradictory at first glance, can be interpreted to suggest that different mechanisms underlie the early development of RCC and the subsequent progression of the disease, and that genetically determined IL-4 may affect these two mechanisms differentially. Thus, a distinct capacity to produce IL-4 may be protective and prevent RCC development, for instance through anti-inflammatory activity, but may be detrimental once the disease is established, for instance by hampering tumor immune surveillance.

For additional exploration of the biologic effects of *IL4* polymorphisms, an LD map (data not shown) was created to detect possible nearby functional variants in strong linkage with our investigated haplotypes. Analysis revealed a high LD throughout the genes *IL4* and *KIF3A*, but no linkage to the nearby locus of *IL13*. Therefore, no linkage of *IL4* haplotypes 1 to 4 to potentially functional *IL13* variants may be expected. *KIF3A* is coding for a subunit of kinesin II, a microtubule-based motility protein.[63] Because of its biologic function, a correlation of *KIF3A* with RCC may not be expected, but should not be excluded either, given that *KIF3A* expression levels are known to play a role in the metastatic ability of tumor cells; its genetic inactivation in mice inhibits renal ciliogenesis and produces polycystic kidney disease.[64,65] To date, there are no reports of association studies of *KIF3A* polymorphisms. In contrast to *KIF3A*, however, association of specific polymorphisms of *IL4* with biologic functions (eg, its transcriptional activity and circulating immunoglobulin E levels) have been shown.[24,25,27,28] Additional detailed genetic investigations inside the LD block harboring *IL4* and *KIF3A* will be needed to reveal and prove the causative polymorphisms correlated with survival in RCC.

IL-4 is a pleiotropic cytokine mainly produced by a subset of $CD4^+$ T cells, designated T helper (TH) 2 cells, but also by basophils, mast cells, natural killer T cells, and γ/δ T cells in response to receptor-mediated activation events.[66] IL-4 plays a central role in regulating the differentiation of antigen-stimulated naive T cells. IL-4 causes such cells to develop into cells capable of producing IL-4 and a series of other cytokines including IL-5, IL-10, and IL-13 (ie, TH2-like cells). Concomitantly, IL-4 powerfully suppresses the appearance of IFN-γ–producing $CD4^+$ T cells (TH1 cells). In addition, IL-4 has been proposed as an anti-inflammatory agent with antitumoral activity.[67] Along this line, previous work has demonstrated growth-inhibitory effects of IL-4 on human RCC in vitro.[10-13] However, clinical administration of IL-4 to patients with RCC or other tumors failed to reproduce the promising in vitro findings in vivo.[15-17] The discrepancy between the in vitro and in vivo effects of IL-4 may again reflect the different mechanisms operating during the early onset of RCC and during disease progression. Thus, enhanced IL-4 production in patients with *IL4* haplotype 1 may inhibit proliferation of RCC in the early phase of the disease, but may cause TH2 deviation during disease progression and thereby prevent effective immune surveillance by TH1 cells.

Reliable prognostic factors in human RCC are Karnofsky performance score[68] and the Eastern Cooperative Oncology Group performance status,[69] which include histopathology of TNM staging and Fuhrman nuclear grading. Less specific and selective prognostic factors are high corrected level of serum calcium, high level of the serum lactate dehydrogenase, and low hemoglobin level.[70,71] Moreover, increased serum IL-6 or serum vascular endothelial growth factor have been reported to influence negatively the prognosis of metastatic RCC.[72] On the basis of the current study, we recommend *IL4* promoter polymorphisms −589/−33 as an additional independent and routine-relevant genetic predictor for survival in metastatic RCC.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The authors indicated no potential conflicts of interest.

AUTHOR CONTRIBUTIONS

Conception and design: Christoph Gassner, Peter Lackner, Martin Thurnher, Reinhold Ramoner
Financial support: Martin Thurnher, Christoph Gassner
Administrative support: Christoph Gassner, Reinhold Ramoner
Provision of study materials or patients: Martin Thurnher, Reinhold Ramoner
Collection and assembly of data: Thomas Kleinrath, Christoph Gassner, Martin Thurnher, Reinhold Ramoner
Data analysis and interpretation: Thomas Kleinrath, Christoph Gassner, Peter Lackner, Martin Thurnher, Reinhold Ramoner
Manuscript writing: Thomas Kleinrath, Christoph Gassner, Peter Lackner, Martin Thurnher, Reinhold Ramoner
Final approval of manuscript: Thomas Kleinrath, Christoph Gassner, Peter Lackner, Martin Thurnher, Reinhold Ramoner

REFERENCES

1. Janzen NK, Kim HL, Figlin RA, et al: Surveillance after radical or partial nephrectomy for localized renal cell carcinoma and management of recurrent disease. Urol Clin North Am 30:843-852, 2003

2. Cohen HT, McGovern FJ: Renal-cell carcinoma. N Engl J Med 353:2477-2490, 2005

3. Thurnher M, Radmayr C, Hobisch A, et al: Tumor-infiltrating T lymphocytes from renal-cell carcinoma express B7-1 (CD80): T-cell expansion by T-T cell co-stimulation. Int J Cancer 62:559-564, 1995

4. Ikemoto S, Yoshida N, Narita K, et al: Role of tumor-associated macrophages in renal cell carcinoma. Oncol Rep 10:1843-1849, 2003

5. Thurnher M, Radmayr C, Ramoner R, et al: Human renal-cell carcinoma tissue contains dendritic cells. Int J Cancer 68:1-7, 1996

6. Ikemoto S, Narita K, Yoshida N, et al: Effects of tumor necrosis factor alpha in renal cell carcinoma. Oncol Rep 10:1947-1955, 2003

7. Alberti L, Thomachot MC, Bachelot T, et al: IL-6 as an intracrine growth factor for renal carcinoma cell lines. Int J Cancer 111:653-661, 2004

8. Nanus DM, Pfeffer LM, Bander NH, et al: Antiproliferative and antitumor effects of alpha-interferon in renal cell carcinomas: Correlation with the expression of a kidney-associated differentiation glycoprotein. Cancer Res 50:4190-4194, 1990

9. Kuebler JP, Oberley TD, Meisner LF, et al: Effect of interferon alpha, interferon beta, and interferon gamma on the in vitro growth of human renal adenocarcinoma cells. Invest New Drugs 5:21-29, 1987

10. Cheon J, Chung DJ, Kim JJ, et al: Inhibitory effects of interleukin-4 on human renal cell carcinoma cells in vitro: In combination with interferon-alpha, tumor necrosis factor-alpha or interleukin-2. Int J Urol 3:196-201, 1996

11. Obiri NI, Hillman GG, Haas GP, et al: Expression of high affinity interleukin-4 receptors on human renal cell carcinoma cells and inhibition of tumor cell growth in vitro by interleukin-4. J Clin Invest 91:88-93, 1993

12. Yu SJ, Kim HS, Cho SW, et al: IL-4 inhibits proliferation of renal carcinoma cells by increasing the expression of p21WAF1 and IRF-1. Exp Mol Med 36:372-379, 2004

13. Falkensammer C, Johrer K, Gander H, et al: IL-4 inhibits the TNF-alpha induced proliferation of renal cell carcinoma (RCC) and cooperates with TNF-alpha to induce apoptotic and cytokine responses by RCC: Implications for antitumor immune responses. Cancer Immunol Immunother 55:1228-1237, 2006

14. Obiri NI, Husain SR, Debinski W, et al: Interleukin 13 inhibits growth of human renal cell carcinoma cells independently of the p140 interleukin 4 receptor chain. Clin Cancer Res 2:1743-1749, 1996

15. Margolin K, Aronson FR, Sznol M, et al: Phase II studies of recombinant human interleukin-4 in advanced renal cancer and malignant melanoma. J Immunother Emphasis Tumor Immunol 15:147-153, 1994

16. Whitehead RP, Unger JM, Goodwin JW, et al: Phase II trial of recombinant human interleukin-4 in patients with disseminated malignant melanoma: A Southwest Oncology Group study. J Immunother 21:440-446, 1998

17. Whitehead RP, Lew D, Flanigan RC, et al: Phase II trial of recombinant human interleukin-4 in patients with advanced renal cell carcinoma: A southwest oncology group study. J Immunother 25:352-358, 2002

18. Motzer RJ, Bander NH, Nanus DM: Renal-cell carcinoma. N Engl J Med 335:865-875, 1996

19. Bidwell JL, Wood NA, Morse HR, et al: Human cytokine gene nucleotide sequence alignments, 1998. Eur J Immunogenet 25:83-265, 1998

20. Bidwell JL, Wood NA, Morse HR, et al: Human cytokine gene nucleotide sequence alignments: Supplement 1. Eur J Immunogenet 26:135-223, 1999

21. Bidwell J, Keen L, Gallagher G, et al: Cytokine gene polymorphism in human disease: On-line databases. Genes Immun 1:3-19, 1999

22. Rao N, Nguyen S, Ngo K, et al: A novel splice variant of interleukin-1 receptor (IL-1R)-associated kinase 1 plays a negative regulatory role in Toll/IL-1R-induced inflammatory signaling. Mol Cell Biol 25:6521-6532, 2005

23. Linker-Israeli M, Wallace DJ, Prehn J, et al: Association of IL-6 gene alleles with systemic lupus erythematosus (SLE) and with elevated IL-6 expression. Genes Immun 1:45-52, 1999

24. Vandenbroeck K, Goris A: Cytokine gene polymorphisms in multifactorial diseases: Gateways to novel targets for immunotherapy? Trends Pharmacol Sci 24:284-289, 2003

25. Rosenwasser LJ, Klemm DJ, Dresback JK, et al: Promoter polymorphisms in the chromosome 5 gene cluster in asthma and atopy. Clin Exp Allergy 25 Suppl 2:74-78, 1995; discussion 95-96

26. Nakashima H, Miyake K, Inoue Y, et al: Association between IL-4 genotype and IL-4 production in the Japanese population. Genes Immun 3:107-109, 2002

27. Kabesch M, Tzotcheva I, Carr D, et al: A complete screening of the IL4 gene: Novel polymorphisms and their association with asthma and IgE in childhood. J Allergy Clin Immunol 112:893-898, 2003

28. Basehore MJ, Howard TD, Lange LA, et al: A comprehensive evaluation of IL4 variants in ethnically diverse populations: Association of total serum IgE levels and asthma in white subjects. J Allergy Clin Immunol 114:80-87, 2004

29. Holtl L, Ramoner R, Zelle-Rieser C, et al: Allogeneic dendritic cell vaccination against metastatic renal cell carcinoma with or without cyclophosphamide. Cancer Immunol Immunother 54: 663-670, 2005

30. Holtl L, Zelle-Rieser C, Gander H, et al: Immunotherapy of metastatic renal cell carcinoma with tumor lysate-pulsed autologous dendritic cells. Clin Cancer Res 8:3369-3376, 2002

31. Louie LG, Hartogensis WE, Jackman RP, et al: Mycobacterium tuberculosis/HIV-1 coinfection and disease: Role of human leukocyte antigen variation. J Infect Dis 189:1084-1090, 2004

32. Mytilineos J, Laux G, Opelz G: Relevance of IL10, TGFbeta1, TNFalpha, and IL4Ralpha gene polymorphisms in kidney transplantation: A collaborative transplant study report. Am J Transplant 4:1684-1690, 2004

33. Hunt PJ, Marshall SE, Weetman AP, et al: Cytokine gene polymorphisms in autoimmune thyroid disease. J Clin Endocrinol Metab 85:1984-1988, 2000

34. Mullighan CG, Marshall SE, Bunce M, et al: Variation in immunoregulatory genes determines the clinical phenotype of common variable immunodeficiency. Genes Immun 1:137-148, 1999

35. Hoffmann SC, Stanley EM, Darrin Cox E, et al: Association of cytokine polymorphic inheritance and in vitro cytokine production in anti-CD3/CD28-stimulated peripheral blood lymphocytes. Transplantation 72:1444-1450, 2001

36. Solovieva S, Leino-Arjas P, Saarela J, et al: Possible association of interleukin 1 gene locus polymorphisms with low back pain. Pain 109:8-19, 2004

37. Bergholdt R, Karlsen AE, Johannesen J, et al: Characterization of polymorphisms of an interleukin 1 receptor type 1 gene (IL1RI) promotor region (P2) and their relation to insulin-dependent diabetes mellitus (IDDM): The Danish Study Group of Diabetes in Childhood. Cytokine 7:727-733, 1995

38. den Dunnen JT, Antonarakis E: Human Genome Variation Society: Nomenclature for the description of sequence variations. http://www.hgvs.org/mutnomen/index.html

39. Hefler LA, Ludwig E, Lebrecht A, et al: Polymorphisms of the interleukin-1 gene cluster and ovarian cancer. J Soc Gynecol Investig 9:386-390, 2002

40. El-Omar EM, Carrington M, Chow WH, et al: Interleukin-1 polymorphisms associated with increased risk of gastric cancer. Nature 404:398-402, 2000

41. Hall SK, Perregaux DG, Gabel CA, et al: Correlation of polymorphic variation in the promoter region of the interleukin-1 beta gene with secretion of interleukin-1 beta protein. Arthritis Rheum 50: 1976-1983, 2004

42. Maksymowych WP, Reeve JP, Reveille JD, et al: High-throughput single-nucleotide polymorphism analysis of the IL1RN locus in patients with ankylosing spondylitis by matrix-assisted laser desorption ionization-time-of-flight mass spectrometry. Arthritis Rheum 48:2011-2018, 2003

43. Matesanz F, Fedetz M, Leyva L, et al: Effects of the multiple sclerosis associated -330 promoter polymorphism in IL2 allelic expression. J Neuroimmunol 148:212-217, 2004

44. Rockman MV, Hahn MW, Soranzo N, et al: Positive selection on a human-specific transcription factor binding site regulating IL4 expression. Curr Biol 13:2118-2123, 2003

45. Caggana M, Walker K, Reilly AA, et al: Population-based studies reveal differences in the allelic frequencies of two functionally significant human interleukin-4 receptor polymorphisms in several ethnic groups. Genet Med 1:267-271, 1999

46. Fishman D, Faulds G, Jeffery R, et al: The effect of novel polymorphisms in the interleukin-6 (IL-6) gene on IL-6 transcription and plasma IL-6 levels, and an association with systemic-onset juvenile chronic arthritis. J Clin Invest 102:1369-1376, 1998

47. Terry CF, Loukaci V, Green FR: Cooperative influence of genetic polymorphisms on interleukin 6 transcriptional regulation. J Biol Chem 275:18138-18144, 2000

48. Shin HD, Winkler C, Stephens JC, et al: Genetic restriction of HIV-1 pathogenesis to AIDS by promoter alleles of IL10. Proc Natl Acad Sci U S A 97:14467-14472, 2000

49. Alamartine E, Berthoux P, Mariat C, et al: Interleukin-10 promoter polymorphisms and susceptibility to skin squamous cell carcinoma after renal transplantation. J Invest Dermatol 120:99-103, 2003

50. Hall MA, McGlinn E, Coakley G, et al: Genetic polymorphism of IL-12 p40 gene in immune-mediated disease. Genes Immun 1:219-224, 2000

51. Pravica V, Perrey C, Stevens A, et al: A single nucleotide polymorphism in the first intron of the human IFN-gamma gene: Absolute correlation with a polymorphic CA microsatellite marker of high IFN-gamma production. Hum Immunol 61:863-866, 2000

52. Tzakas P, Wong BY, Logan AG, et al: Transforming growth factor beta-1 (TGFB1) and peak bone mass: Association between intragenic polymorphisms and quantitative ultrasound of the heel. BMC Musculoskelet Disord 6:29-39, 2005

53. Kim YJ, Lee HS, Yoon JH, et al: Association of TNF-alpha promoter polymorphisms with the clearance of hepatitis B virus infection. Hum Mol Genet 12:2541-2546, 2003

54. Lu CC, Sheu BS, Chen TW, et al: Host TNF-alpha-1031 and -863 promoter single nucleotide polymorphisms determine the risk of benign ulceration after *H. pylori* infection. Am J Gastroenterol 100:1274-1282, 2005

55. The International HapMap Project: http://www.hapmap.org. Nature 426:789-796, 2003

56. Lo YM, Patel P, Newton CR, et al: Direct haplotype determination by double ARMS: Specificity, sensitivity and genetic applications. Nucleic Acids Res 19:3561-3567, 1991

57. Hardy G: Mendelian proportions in a mixed population. Science 28:49-50, 1908

58. Benjamini Y, Drai D, Elmer G, et al: Controlling the false discovery rate in behavior genetics research. Behav Brain Res 125:279-284, 2001

59. Sauerbrei W, Schumacher M: A bootstrap resampling procedure for model building: Application to the Cox regression model. Stat Med 11:2093-2109, 1992

60. Beghé B, Barton S, Rorke S, et al: Polymorphisms in the interleukin-4 and interleukin-4 receptor alpha chain genes confer susceptibility to asthma and atopy in a Caucasian population. Clin Exp Allergy 33:1111-1117, 2003

61. Modi WS, O'Brien TR, Vlahov D, et al: Haplotype diversity in the interleukin-4 gene is not associated with HIV-1 transmission and AIDS progression. Immunogenetics 55:157-164, 2003

62. Ries LAG, Eisner MP, Kosary CL, et al: National Cancer Institute: SEER Cancer Statistics Review, 1975-2002. http://seer.cancer.gov/csr/1975_2002/

63. Vale RD: The molecular motor toolbox for intracellular transport. Cell 112:467-480, 2003

64. Yanagawa T, Watanabe H, Takeuchi T, et al: Overexpression of autocrine motility factor in metastatic tumor cells: Possible association with augmented expression of KIF3A and GDI-beta. Lab Invest 84:513-522, 2004

65. Lin F, Hiesberger T, Cordes K, et al: Kidney-specific inactivation of the KIF3A subunit of kinesin-II inhibits renal ciliogenesis and produces polycystic kidney disease. Proc Natl Acad Sci U S A 100:5286-5291, 2003

66. Nelms K, Keegan AD, Zamorano J, et al: The IL-4 receptor: Signaling mechanisms and biologic functions. Annu Rev Immunol 17:701-738, 1999

67. Banchereau J, Briere F, Galizzi JP, et al: Human interleukin 4. J Lipid Mediat Cell Signal 9:43-53, 1994

68. Zisman A, Pantuck AJ, Dorey F, et al: Improved prognostication of renal cell carcinoma using an integrated staging system. J Clin Oncol 19:1649-1657, 2001

69. Oken MM, Creech RH, Tormey DC, et al: Toxicity and response criteria of the Eastern Cooperative Oncology Group. Am J Clin Oncol 5:649-655, 1982

70. Motzer RJ, Mazumdar M, Bacik J, et al: Survival and prognostic stratification of 670 patients with advanced renal cell carcinoma. J Clin Oncol 17:2530-2540, 1999

71. Motzer RJ, Bacik J, Schwartz LH, et al: Prognostic factors for survival in previously treated patients with metastatic renal cell carcinoma. J Clin Oncol 22:454-463, 2004

72. Negrier S, Perol D, Menetrier-Caux C, et al: Interleukin-6, interleukin-10, and vascular endothelial growth factor in metastatic renal cell carcinoma: Prognostic value of interleukin-6–from the Groupe Francais d'Immunotherapie. J Clin Oncol 22:2371-2378, 2004

Acknowledgment

We thank Hubert Gander and Andrea Rahm for their efforts in sample collection. We also thank G. Bartsch, head of the Department of Urology, as well as D. Schönitzer, former head of the Central Institute for Blood Transfusion, for their continuous support.