Original Article

Roles of Loss of Chromosome 14q Allele in the Prognosis of Renal Cell Carcinoma with C-reactive Protein Abnormity

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Abstract

Background: Renal cell carcinoma (RCC) is frequently associated with paraneoplastic inflammatory syndrome (PIS). This study aimed at exploring the connections between the survival rate and specific gene alterations and the potential mechanism.
Methods: We retrospectively studied 69 surgical RCC cases from August 2014 to February 2016, including 18 cases of clear cell RCC (ccRCC) demonstrating elevated pretreatment serum C-reactive protein (CRP, Group A). Twelve of the 18 cases were symptomized with febrile episode. We also selected 49 cases of ccRCC with normal pretreatment CRP (Group B). Using 22 microsatellite markers, we compared the incidence of loss of heterozygosity (LOH) between Group A and Group B. All statistical tests are two-sided.
Results: The 3p LOH was common in both Group A (89%) and Group B (92%). The frequency of 14q LOH in Group A (16 of 18) was higher than Group B (4 of 49, $\chi^2 = 40.97$ $P < 0.0001$). The 3p and 14q LOH were the characteristics of ccRCC with elevated acute phase reactants, including PIS, regardless of the presence of metastasis. On the contrary, 14q LOH was a rare genomic alternation in advanced-staged ccRCC without PIS. The overall survival of patients with elevated CRP (33.3%) was lower than its counterparts (6.1%, hazard ratio=1.852, $P < 0.0001$) in Kaplan-Meier curve.
Conclusions: The results imply that the disruption of a 14q gene(s) might result in not only the inflammatory manifestations in the tumor host but also the poor survival rate as well. The isolation of the gene(s) on 14q might be a vital goal in the treatment of PIS-associated RCC.

Key words: C-reactive Protein; Gene Alterations; Loss of Chromosome; Paraneoplastic Inflammatory Syndrome; Renal Cell Carcinoma

Introduction

The importance of understanding the pathophysiology and biology of many paraneoplastic syndromes associated with renal cell carcinoma (RCC) lies in the fact that these protean symptoms might be the initial presentation of some either primary or recurrent diseases. RCC is known to be frequently associated with paraneoplastic inflammatory syndrome (PIS), including episodes of fever, increased serum acute phase protein levels, and body weight loss. Elevated inflammatory parameters have been observed in patients with or without distant metastasis.[1] These inflammatory markers are recognized as poor prognostic signs[2] and predictions of poor responses to cytokine therapy.[3] The molecular mechanisms responsible for the symptoms of PIS in RCC remain poorly understood although previous reports suggested that endogenous interleukin 6 (IL-6) production in RCC might be playing a key role.[4]

Here, we compare loss of heterozygosity (LOH) in clear cell RCC (ccRCC) between the ones with and without PIS. The results show that 3p and 14q LOH are common in the RCC

Access this article online

Quick Response Code:

Website: www.cmj.org

DOI: 10.4103/0366-6999.213962

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Received: 28-07-2017 **Edited by:** Peng Lyu
How to cite this article: Wang G, Zhang DM, Zhuang HY, Yin C, Liu J, Wang ZC, Cai LC, Ren MH, Xu WH, Zhang C. Roles of Loss of Chromosome 14q Allele in the Prognosis of Renal Cell Carcinoma with C-reactive Protein Abnormity. Chin Med J 2017;130:2176-82.

group with PIS, regardless of tumor stage, while a ccRCC subgroup without PIS carries 3p LOH frequently and 14q LOH infrequently. The present data support the idea that distinct genomic alterations of ccRCC, especially those in chromosome 14q, might contribute to the presence of elevated acute phase reactants, including paraneoplastic febrile episode in ccRCC. This study discusses the possible mechanisms of 14q LOH contributing to the paraneoplastic inflammatory phenomenon in ccRCC and their biological significance.

Methods

Ethical approval

As a retrospective study, since the data analyses are performed anonymously, this study is exempt from ethical approval and patient informed consent.

Tissue samples and histological examination

Paired blood/tumor samples and complete clinical data were surgically gathered from 69 sporadic RCC cases, which were collected from The First Affiliated Hospital of Harbin Medical University from August 2014 to February 2016. None of the patients had undergone preoperative chemotherapy or immunotherapy. Serum C-reactive protein (CRP, normal values <6 mg/L) and erythrocyte sedimentation rate (ESR, normal values <30 mm/h) were analyzed before nephrectomy. The presence of paraneoplastic fever (defined as >37.5°C) and body weight loss (defined as >1 kg/month in previous 6 months) was also assessed before treatment. For each surgically removed tissue, a 5-mm diameter specimen of necrotic RCC was bisected as a representative, with one of the two halves frozen and stored immediately at −80°C until subsequent analyses. The remaining material was fixed with buffered formalin for histopathological diagnosis, allowing us to select samples containing at least 75% tumor cells. Histopathological evaluation and grading were performed according to the protocol of Thoenes *et al.*[5] and in accordance with the classification recently reestablished by the Union for International Cancer Control (UICC) and the American Joint Committee on Cancer.[6] Tumor-node-metastasis stages were determined using the criteria currently established by the UICC.[7]

Patients and samples selection

The medical records of the 69 RCC patients were analyzed retrospectively, among which 18 cases demonstrated elevated pretreatment serum CRP (mean pretreatment CRP ± standard deviation [SD]: 103.3 ± 42.5 mg/L) and were designated as Group A. Tumornodemetastasis stages are shown using the currentlyestablished criteria according to the UICC. The clinical characteristics of Group A, including ESR values, paraneoplastic febrile episode, and weight loss, are depicted in Table 1. Histological examination revealed that all Group A samples contained clear cell carcinoma components. Forty-nine ccRCC cases with normal pretreatment CRP (mean pretreatment

Table 1: Clinicopathological characteristics of RCC With elevated pretreatment CRP (Group A)

Code	Age (years)*	Sex	Cell type	Structure	Grade	Tumor size (cm)	Stage†	CRP (mg/L)‡	ESR (mm)§	Paraneoplastic fever‖	Weight loss¶
A-1	51	Male	Clear with sarcomatoid	Solid	3	12	IV	130	111	No	Yes
A-2	74	Male	Clear	Alveolar	2	8.4	II	152	150	No	Yes
A-3	65	Male	Clear	Alveolar	2	8	III	116	Scale over	Yes	Yes
A-4	64	Male	Clear	Alveolar	2	12.5	IV	120	140	No	Yes
A-5	82	Male	Clear	Alveolar	2	6	IV	97	Scale over	Yes	Yes
A-6	73	Female	Clear	Alveolar	1	8	II	157**	150	Yes††	Yes
A-7	54	Male	Clear	Alveolar	2	5	I	100**	120	Yes††	Yes
A-8	70	Male	Clear	Alveolar	2	6	I	48**	100	Yes††	Yes
A-9	73	Male	Clear	Alveolar	3	7	III	65	80	No	Yes
A-10	67	Male	Clear	Alveolar	2	5	IV	107	138	No	No
A-11	69	Male	Clear and granular	Alveolar	1	8	IV	190	Scale over	Yes	Yes
A-12	48	Male	Clear	Alveolar	2	7	IV	93	NA	Yes	No
A-13	73	Male	Clear and granular	Alveolar	2	7.5	III	38	123	Yes	Yes
A-14	73	Male	Clear	Alveolar	2	8	IV	122	101	Yes	Yes
A-15	49	Female	Clear	Alveolar	3	13.5	IV	79	85	No	No
A-16	58	Female	Clear	Alveolar	1	5	I	42**	NA	Yes††	No
A-17	61	Male	Clear	Alveolar	2	6.5	IV	82	92	Yes	No
A-18	74	Male	Clear	Alveolar	3	9	IV	142	Scale over	Yes	Yes

*The mean age at diagnosis of Group A RCCs was 65 years (range, 48–82 years) including males (n = 15) and females (n = 3); †Tumor-node-metastasis stages are shown using the currently-established criteria according to the UICC; ‡Level of CRP before treatment (normal <6 mg/L). CRP in Group A was 103.3 ± 42.5 mg/L (mean CRP ± SD). The entire Group A patients were confirmed to have "tumor-related CRP elevation" by ruling out other inflammatory factors such as infection or arthritis. The workup included evaluation of medical history, routine blood tests, chest X-ray, and urinalysis. The entire Group A patients had normal leukocytes counts before treatment; §Pretreatment erythrocyte sedimentation rate at 1 h (normal <30 mm/h). The mean level of erythrocyte sedimentation rate in Group A was not calculated since four cases showed scale over for erythrocyte sedimentation rate at 1 h. NA; erythrocyte sedimentation rate was not assessed; ‖Charts were reviewed to determine the presence of paraneoplastic fever, defined as >37.5°C for more than 3 consecutive days; ¶Charts were reviewed to determine the presence of pretreatment weight loss, defined as >1 kg/month in previous 6 months; **CRPs were normalized after nephrectomy; ††Pretreatment paraneoplastic febrile episode disappeared after nephrectomy. RCC: Renal cell carcinoma; CRP: C-reactive protein; ESR: Erythrocyte sedimentation rate; SD: Standard deviation; UICC: Union for International Cancer Control; NA: Not available.

CRP ± SD: 2.0 ± 1.7 mg/L) were selected from the series above and designated as Group B. This group had neither history of paraneoplastic fever nor body weight loss before nephrectomy. The clinicopathological data of

Table 2: Clinicopathological characteristics of RCC with normal pretreatment CRP* (Group B)

Code	Age (years)†	Sex	Cell type	Structure	Grade	Tumor size (cm)	Stage
B-1	67	Female	Clear	Alveolar	2	12	II
B-2	52	Male	Clear	Alveolar	2	4	I
B-3	73	Male	Clear	Alveolar	1	2	I
B-4	72	Male	Clear	Alveolar	1	5	I
B-5	46	Male	Clear	Alveolar	2	3	I
B-6	65	Male	Clear	Tubular	2	5	I
B-7	70	Male	Clear	Alveolar	2	7.5	II
B-8	39	Male	Clear	Cystic	2	11	II
B-9	67	Female	Clear	Alveolar	1	3	I
B-10	76	Female	Clear	Alveolar	2	3	I
B-11	66	Male	Clear	Alveolar	2	3	I
B-12	65	Male	Clear	Alveolar	1	5	I
B-13	33	Male	Clear	Alveolar	2	8	II
B-14	43	Male	Clear	Alveolar	1	4	I
B-15	41	Male	Clear	Alveolar	2	3	I
B-16	52	Male	Clear	Alveolar	2	5	I
B-17	63	Female	Clear	Alveolar	1	7	I
B-18	78	Female	Clear	Alveolar	1	6	I
B-19	81	Male	Clear	Alveolar	1	10	II
B-20	80	Female	Clear	Alveolar	1	4	I
B-21	62	Female	Clear	Alveolar	2	4	I
B-22	65	Male	Clear	Alveolar	2	5	I
B-23	50	Male	Clear	Alveolar	1	3	I
B-24	75	Female	Clear	Alveolar	2	11	II
B-25	49	Female	Clear	Alveolar	2	6	I
B-26	73	Female	Clear	Alveolar	2	8	II
B-27	70	Male	Clear	Alveolar	1	6	I
B-28	79	Female	Clear	Alveolar	1	3	I
B-29	77	Male	Clear	Alveolar	2	6	I
B-30	59	Female	Clear	Alveolar	1	5	I
B-31	70	Male	Clear	Alveolar	1	4	I
B-32	64	Male	Clear	Alveolar	2	4	I
B-33	76	Male	Clear	Alveolar	1	6	III
B-34	67	Male	Clear	Alveolar	2	11	III
B-35	72	Male	Clear	Alveolar	3	8	IV
B-36	59	Male	Clear and granular	Alveolar	2	12	IV
B-37	70	Male	Clear	Alveolar	2	8	IV
B-38	69	Female	Clear	Alveolar	2	10	III
B-39	64	Male	Clear	Alveolar	2	8	III
B-40	60	Male	Clear	Alveolar	2	10	III
B-41	63	Male	Clear	Alveolar	2	7	IV
B-42	57	Male	Clear	Alveolar	2	6	IV
B-43	55	Male	Clear and granular	Alveolar	2	8	III
B-44	49	Female	Clear	Alveolar	3	7.5	IV
B-45	58	Female	Clear	Alveolar	2	11	IV
B-46	75	Male	Clear and granular	Alveolar	2	7	IV
B-47	59	Male	Clear with sarcomatoid	Solid	3	14	IV
B-48	72	Male	Clear	Alveolar	2	9	III
B-49	61	Female	Clear	Alveolar	2	11	IV

*The entire Group B patients demonstrated normal pretreatment CRP level (CRP <6 mg/L, mean ± SD = 2.0 ± 1.7 mg/L, n = 49). Pretreatment erythrocyte sedimentation rate at 1 h in Group B patients was 13.9 ± 8.2 mm (mean ± SD, n = 43). The entire Group B patients had neither history of paraneoplastic fever nor body weight loss before nephrectomy; †The mean age at diagnosis of Group B RCCs was 63 years (range, 33–81 years) including males (n = 33) and females (n = 16); ‡Tumor-node-metastasis stages are shown by using currently established criteria according to the UICC. RCC: Renal cell carcinoma; CRP: C-reactive protein; ESR: Erythrocyte sedimentation rate; SD: Standard deviation; UICC: Union for International Cancer Control.

Group B are presented in Table 2. One ccRCC case with normal pretreatment CRP and elevated pretreatment ESR was excluded from this study (CRP: 5 mg/L and ESR: 58 mm/h). Another ccRCC case showed pretreatment CRP elevation (CRP: 15 mg/L) and was suggestive of coexistent viral infection. This case was excluded from this study because CRP elevation was not judged to be "tumor related." We also excluded three cases of papillary RCC with normal pretreatment CRP from this study.

Selection of microsatellite markers

Twenty-two polymorphic microsatellite markers from eight chromosomal regions were selected for microsatellite analyses. To avoid the stutter bands in polymerase chain reaction (PCR) products, we used tri- or tetra-nucleotide microsatellite markers, except for D3S1300. Primers sequences and locations were obtained from the Cooperative Human Linkage Center (http://www.chlc.org/ChlcIntegratedMaps.html). Analysis of 3p allele loss was assessed with six different microsatellite markers. The LOH analyses at 4q, 6q, 7q, 8p, 9p, 14q, and 17p were performed with at least two polymorphic microsatellite markers so that more than 90% of the analyzed cases were informative at each analyzed chromosomal arm. One primer of each primer pair was fluorescein-labeled at the 5'-end for the subsequent microsatellite analysis performed on an automated laser-activated fluorescent DNA sequencer.

DNA isolation and polymerase chain reaction

Tumor fragments were homogenized in the presence of liquid nitrogen and incubated in 10 mmol/L Tris-HCl (pH 8.0), 50 mmol/L ethylenediaminetetraacetic acid, 10 mmol/L NaCl, 2% N-lauroyl sarcosine, and 200 g/ml proteinase K (Boehringer Mannheim GmbH, Mannheim, Germany) for 20 h at 42°C, followed by phenol chloroform extraction and ethanol precipitation. Peripheral blood lymphocyte DNA was extracted by QIAamp DNA Blood Mini Kit (QIAGEN, Valencia, CA, USA). PCRs were carried out in 20 tubes with each containing 12.5 pmol primer pairs. Genomic DNA from blood cells or tumors (20–50 ng) was used as a template for amplification (30 cycles of denaturation for 1 min at 94°C, annealing for 1 min at 55°C, and extension for 2 min at 72°C for all the primer pairs) on a Peltier Thermal Cycler-200 (MJ Research, San Francisco, CA, USA).

Polymerase chain reaction-based loss of chromosome assays

PCR products were separated electrophoretically on a 5% polyacrylamide gel and detected by laser fluorescence with an automated sequencer (ALF, Pharmacia, Biotech). Fluorescent gel data were collected automatically during electrophoresis and calculated with Fragment Manager 1.1 software (Amersham Pharmacia Biotech, NY, USA), which provided peak size, height, and area under the curve. PCR products from normal blood cells and the corresponding tumor tissue were analyzed on the same gel. Automatic analyzing of peak areas allowed for the relative quantification of PCR products and the determination of LOH ratios as described previously.[8] Briefly, LOH ratios were calculated by inserting the tumor and normal allele intensities into the following formula: (tumor allele 1/tumor allele 2)/(normal allele 1/normal allele 2). LOH was scored when the intensity ratio equaled either <0.70 (tumor allele 1 LOH) or >1.4 (tumor allele 2 LOH). The chosen level (*i.e.*, 75%) of the enrichment of targeted tumor cells was adequate to meet or exceed this 1.4-fold comparative ratio in the specimens with LOH.[8] The LOH assays were repeated at least twice to confirm the results.

Statistical analysis

Statistical differences of chromosomal locus between the two groups were examined by the use of Fisher's exact test. Clinical characteristics were compared using the *Z*-test and Fisher's exact test. Overall survival (OS) rate was estimated with the Kaplan-Meier method and compared by the log-rank test. All statistical analyses were carried out using SPSS 16.0 statistical software package (SPSS, Chicago, IL, USA). A $P < 0.05$ was considered statistically significant.

Results

Clinical characteristics of patients with renal cell carcinoma

The distinction and the association between clinicopathological parameters and preoperative CRP levels in the 69 patients are shown in Table 3. The patients with elevated preoperative CRP levels were older than the ones with normal preoperative CRP level [Table 3, $t = 3.919$ $P < 0.001$].

The overall survival in clear cell renal cell carcinoma with or without elevated pretreatment C-reactive protein

We divided the 69 patients into two groups based on the level of CRP (normally values <6 mg/L or not). OS rates were been studied in Group A and Group B. The OS of patients with elevated CRP (33.3%) was lower than its counterparts (6.1%) in Kaplan-Meier curve [Figure 1, $P < 0.0001$]. On our survey, six patients died of RCC, while two died of other causes.

Loss of chromosome analysis at 3p in clear cell renal cell carcinoma with or without elevated pretreatment C-reactive protein

We initially conducted LOH analysis at chromosome 3p to determine whether ccRCC with symptoms of PIS,

Table 3: Clinical characteristics of RCC patients

Parameters	CRP ≥6 mg/L ($n = 18$)	CRP <6 mg/L ($n = 43$)	t/χ^2	*P*
Age (years)	66.06 ± 14.38	54.87 ± 9.76	3.919	<0.001
Sex: Male	15 (83.3)	33 (55.9)	1.656	0.237
Side: Right	9 (50.0)	31 (52.5)	0.963	0.403
ECOG-PS	18.00 ± 0.56	63.00 ± 0.21	1.731	0.028
BMI (kg/m^2)	24.06 ± 3.56	24.84 ± 3.58	0.094	0.416
Hypertension	5 (27.8)	15 (25.4)	0.051	1.000
Diabetes mellitus	1 (5.6)	7 (11.9)	0.954	0.433

Data are presented as *n* (%) or mean ± SD. RCC: Renal cell carcinoma; CRP: C-reactive protein; BMI: Body mass index; ECOG-PS: Eastern Cooperative Oncology Group Performance Status; SD: Standard deviation.

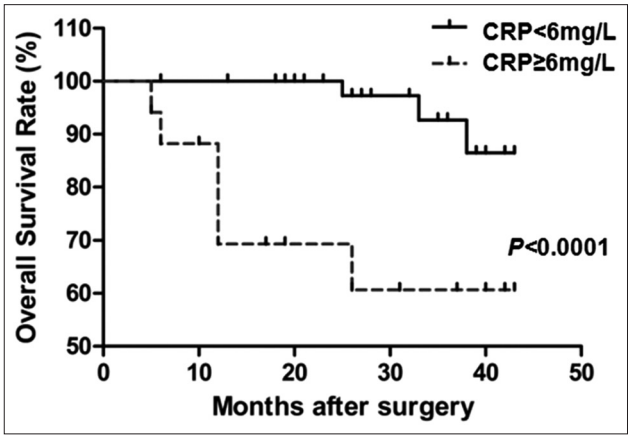

Figure 1: Kaplan-Meier analysis of overall survival for Group A and Group B (hazard ratio=1.852, $P < 0.0001$). CRP: C-reactive protein.

including elevated pretreatment CRP (Group A), had distinct 3p deletion patterns from non-PIS RCC (Group B). The frequency of LOH is summarized using six microsatellite markers at chromosome 3p loci in Table 4. The results showed that LOH at 3p was common in both Group A (89%) and Group B (92%). Markers D3S2387, D3S3030, D3S1289, D3S1766, D3S1300, and D3S2406 detection revealed no significant difference of LOH in Groups A and B [Table 4].

Loss of chromosome analyses at 4q, 6q, 7q, 8p, 9p, 14q, and 17p in clear cell renal cell carcinoma with or without elevated pretreatment C-reactive protein

Due to the result that 3p LOH was a common event in ccRCC, regardless of the presence of PIS symptoms, including elevated pretreatment CRP, we analyzed chromosomal locus other than 3p to determine whether additional chromosomal deletions were associated with PIS in ccRCC. We conducted LOH analysis at 4q, 6q, 7q, 8p, 9p, 14q, and 17p in ccRCC using 16 microsatellite markers for these chromosomal regions and compared the frequency of LOH between Groups A and B. The rate of LOH at each locus is summarized in Table 5. The frequency of 14q LOH in Group A was higher than that in Group B at D14S1426, D14S617, D14S611, and D14S611 [Table 5, $\chi^2 = 31.51$, $\chi^2 = 35.12$, $\chi^2 = 38.56$, and $\chi^2 = 27.75$, respectively, all $P < 0.0001$].

Discussion

Fever is noted in 20–30% patients with RCC, which is often regarded as a presenting symptom.[9,10] Fever occasionally disappears after nephrectomy and might recur when metastasis advances.[9] The elevation of inflammatory parameters such as CRP and ESR has already been confirmed in RCC patients with or without metastasis.[11] IL-6 is a cytokine expressed in RCC which derives cell lines, and it is associated with the symptoms of PIS, including the stimulation of acute phase reactants in RCC. IL-6 acts as an endogenous pyrogen, inducing the expression of acute phase protein genes such as the *CRP* gene.[12,13] Indeed, a previous report showed that the levels of CRP and IL-6 are correlated in RCC, suggesting that IL-6 is involved in CRP elevation in this disease.[14] However, it still remains unclear why a certain group of RCC is associated with inflammatory signs, including CRP and IL-6 elevation. Given that RCC patients with inflammatory manifestations are suggestive of poor prognosis and poor response to cytokine therapy, it is important to search for the tumor-specific gene alterations, which might lead to

Table 4: LOH at chromosome 3p in ccRCC with and without elevated pretreatment CRP, *n/N*

Chromosomal locus*	Group A ($n = 18$)†	Group B ($n = 49$)†	χ^2	P‡
D3S2387/3pter	13/15	42/43	2.750	0.16
D3S3030/3p25	9/12	29/31	2.900	0.12
D3S1289/3p21	13/14	30/38	1.380	0.41
D3S1766/3p14	12/14	36/40	0.190	0.64
D3S1300/3p14	11/13	34/40	0.001	1.00
D3S2406/3p12	12/17	28/40	0.002	1.00
Any LOH at 3p	16/18	45/49	0.140	0.65

*While their precise order and locus are controversial, the markers are arranged in order from telomeric to centromeric; †Number of LOH/number of informative cases (% LOH); ‡Difference in incidence of LOH between Group A and Group B was analyzed using Fisher's exact test. All P values are two-sided. LOH: Loss of chromosome; ccRCC: Clear cell renal cell carcinoma.

Table 5: LOH at 4q, 6q, 7q, 8p, 9p, 14q, and 17p in ccRCC with and without elevated pretreatment CRP, *n/N*

Chromosomal locus*	Group A† ($n = 18$)	Group B† ($n = 49$)	χ^2	P‡
4S2417	3/12	10/37	0.02	1.00
4S2374	2/13	11/41	0.71	0.48
Any LOH at 4q	3/15	14/47	0.55	0.53
6S1027	4/13	11/39	0.03	1.00
6S1273	4/13	9/35	0.12	0.73
Any LOH at 6q	5/17	13/49	0.05	0.75
7S1804	9/15	12/42	4.69	0.06
7S1801	8/15	10/36	3.03	0.11
Any LOH at 7q	9/17	16/48	2.04	0.25
8S1130	4/11	12/38	0.09	1.00
8S1109	5/14	17/39	0.26	0.76
Any LOH at 8p	5/17	17/46	0.31	0.77
9S925	5/14	7/38	1.72	0.27
9S921	4/11	8/35	0.79	0.44
Any LOH at 9p	7/16	10/45	2.72	0.12
14S1426	13/14	3/34	31.51	<0.001
14S617	13/15	2/38	35.12	<0.001
14S611	11/12	2/42	38.56	<0.001
14S616	9/12	2/41	27.75	<0.001
Any LOH at 14q	16/18	4/49	40.97	<0.001
17S1288	3/14	2/38	3.08	0.11
17S185	2/13	3/40	0.71	0.59
Any LOH at 17p	3/17	4/47	1.07	0.37

*While their precise order and locus are controversial, the markers are arranged in order from telomeric to centromeric; †Number of LOH/number of informative cases (% LOH); ‡Difference in incidence of LOH between Group A and Group B was analyzed using Fisher's exact test. All P values are two-sided. LOH: Loss of chromosome; ccRCC: Clear cell renal cell carcinoma.

the identification of the gene(s) directly contributing to the development of PIS in RCC.

As known, no previous study has explored specific gene alterations in RCC-associated PIS. From our initial histopathological observations, we hypothesized that certain type(s) of gene alterations in ccRCC contribute to PIS. We first compared the LOH frequency on chromosome 3p regions between Groups A and B. Until now, at least three different tumor suppressor genes seem to be involved in ccRCC tumorigenesis;[15-18] (1) 3p12-14, which includes the breakpoint of the familial t(3;8) constitutional translocation and a recently cloned, putative tumor suppressor gene, the fragile histidine triad gene, is involved in hereditary RCC development; (2) 3p21.2-21.3, a common region of deletion in many cancers including lung cancer; and (3) 3p25-26, which contains tumor suppressor gene of von Hippel–Lindau disease.[17,19,20] No significant difference in LOH frequency was detected among the six different 3p markers between Groups A and B, indicating that chromosomal deletion on 3p is a common lesion in ccRCC regardless of the presence of PIS.

Next, we analyzed other chromosomal loci to see whether additional chromosomal LOH other than 3p LOH might be required for the development of ccRCC with PIS. Sixteen additional polymorphic microsatellite markers for seven chromosomal arms were selected on the basis of previous reports in the literature describing of LOH in ccRCC.[16,21-23] LOH at 8p, 9p, and 14q has been reportedly associated with advanced-stage ccRCC.[24] In agreement with the previous reports, we identified high frequencies of 8p, 9p, and 14q LOH in the advanced-stage ccRCC samples (data not shown). Wu *et al*.[25] demonstrated that 14q loss in ccRCC was correlated with poor patient outcome. Among all the chromosome arms we investigated, only 14q loss was specifically observed more frequent in ccRCC with PIS than ccRCC without PIS. The difference between Group A and Group B was statistically different (all $P < 0.0001$) at D14S1426, D14S617, D14S611, and D14S611. Furthermore, 5/5 organ-confined cases of Group A showed 14q loss at any site of D14S1426, D14S617, and D14S611. They indicate that 14q loss might be a key genomic change leading to the development of PIS in ccRCC, regardless of tumor stage. It should be noted that the present study neither have cover the entire chromosomal arms being studied nor can the possibility be excluded that other chromosomal alterations specific to PIS might exist in RCC. The present results imply that disruption of 14q gene(s) might result in not only aggressive tumor growth but also in the development of inflammatory syndromes. In agreement with this hypothesis, other types of malignancies (e.g., malignant mesothelioma, Hodgkin's lymphoma, and nasopharyngeal cancer) have been reportedly showing high incidence of paraneoplastic fever,[26] IL-6 production,[27] and involvement of 14q alterations.[28] Although the association between inflammatory manifestations and 14q alterations in these malignancies has not been tested, recent studies have implied that RCC-derived soluble factors might compromise the antitumor immune system in RCC patients, suggesting that RCC itself can produce critical factors which impair the host immune system.[29,30] It is intriguing to speculate that the putative tumor-derived factors might be attributing to 14q LOH in RCC with PIS.

In conclusion, we have clarified genomic features of RCC with PIS. The results imply that the disruption of a 14q gene(s) might result in not only aggressive tumor growth but also inflammatory manifestations in the tumor host as well. We will like to believe that targeting the gene(s) on 14q constitute might bring a promising future to the gene therapy for PIS-associated RCC.

Financial support and sponsorship

This work was supported by grants from the National Natural Science Foundations of China (No. 81171996, and No. 81272289).

Conflicts of interest

There are no conflicts of interest.

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