Int J Clin Exp Pathol 2015;8(4):3636-3647
www.ijcep.com /ISSN:1936-2625/IJCEP0006595

Original Article

Chromosomal imbalances revealed in primary renal cell carcinomas by comparative genomic hybridization

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Received February 2, 2015; Accepted March 30, 2015; Epub April 1, 2015; Published April 15, 2015

Abstract: Renal cell carcinoma (RCC) accounts for approximately 3% of all new cancer cases. Although the classification of RCC is based mainly on histology, this method is not always accurate. We applied comparative genomic hybridization (CGH) to determine genomic alterations in 46 cases of different RCC histological subtypes [10 cases of clear cell RCC (CCRCC), 13 cases of papillary RCC (PRCC), 12 cases of chromophobe RCC (CRCC), 9 cases of Xp11.2 translocation RCC (Xp11.2RCC), 2 cases of undifferentiated RCC (unRCC)], and investigated the relationships between clinical parameters and genomic aberrations. Changes involving one or more regions of the genome were seen in all RCC patients; DNA sequence gains were most frequently (>30%) seen in chromosomes 7q, 16p, and 20q; losses from 1p, 3p, 13q, 14q, and 8p. We conclude CGH is a useful complementary method for differential diagnosis of RCC. Loss of 3p21-25, 15q, and gain of 16p11-13 are relatively particular to CCRCC vs. other types of RCC. Gain of 7p13-22, 8q21-24, and loss of 18q12-ter, 14q13-24, and Xp11-q13/Y are more apparent in PRCC, and gain of 8q21-24 is characteristic of type 2 PRCC vs. type 1 PRCC. Loss of 2q12-32, 10p12-15, and 11p11-15, 13p are characteristic of CRCC, and gain of 3p and loss of 11p11-15 and 13p are significant differentiators between common CRCC and CRCC accompanied by sarcomatous change groups. Gain of Xp11-12 is characteristic of the Xp11.2RCC group. Based on Multivariate Cox regression analysis, aberration in 5 chromosome regions were poor prognostic markers of RCC, and include the gain of chromosome 12p12-ter (P = 0.034, RR = 3.502, 95% CI 1.097-11.182), 12q14-ter (P = 0.002, RR = 5.115, 95% CI 1.847-14.170), 16q21-24 (P = 0.044, RR = 2.629, 95% CI 1.027-6.731), 17p12-ter (P = 0.017, RR = 3.643, 95% CI 1.262-10.512) and the loss of 18q12-23 (P = 0.049, RR = 2.911, 95% CI 1.006-8.425), which may provide clues of new genes involved in RCC tumorigenesis.

Keywords: Renal cell carcinoma, comparative genomic hybridization, chromosomal change

Introduction

Renal cell carcinoma (RCC) accounts for approximately 3% of all new cancer cases and incidence rates have been steadily grown over the last 3 decades [1-3]. About one-third of patients present with metastatic disease at initial diagnosis and surgical resection remain the only curative therapy for RCC; however, up to 50% of patients undergoing nephrectomy for clinically localized RCC will develop local recurrence or distant metastasis. Over all stages, nearly 50% of patients die within 5 years of diagnosis [4-6]. As in most cancers, clinical variables play a major role in prognosis of localized RCC, but accumulation of genetic aberrations is central to the initiation and prognosis of RCC and other cancers. Therefore, integration of genetic markers into traditional approaches may allow a more accurate prediction of prognosis [7, 8].

RCC consists of a heterogeneous group of epithelial tumors with different histological features. There are 4 main subtypes of renal cell carcinoma associated with distinct clinical outcomes and classified according to their histopathology: clear cell RCC (CCRCC), papillary RCC (PRCC), chromophobe RCC (CRCC), and the recently recognized rare renal carcinoma asso-

ciated with Xp11.2 translocation/TFE3 gene fusions (Xp11.2RCC). Although the classification of RCC is based mainly on histology, the morphology of RCC subtypes is sometimes similar, so the method is not always accurate. The WHO classification has introduced genetic alterations as a hallmark corresponding to the histologic subtypes of RCC. We applied comparative genomic hybridization (CGH) to identify the genomic alterations in 46 cases of different histological subtypes of renal cell carcinoma. We analyzed correlations between chromosome aberrations and clinicopathological variables, including tumor stage and nuclear grade, and validated the use of CGH for differential diagnosis of RCCs.

Materials and methods

Ethics statement

Ethical approval was obtained from Institutional Ethics Review Board (IERB), The First Affiliated Hospital, Shihezi University School of Medicine and all participants provided written informed consent for themselves.

Primary tumor

Forty-six methanol-fixed, paraffin-embedded primary renal cell carcinoma samples were retrieved from the archives of the Department of Pathology, Shihezi University School of Medicine of Xinjiang, China. There were 5 subtypes of RCC: 10 cases of CCRCC, 13 cases of PRCC (6 cases of type 1 PRCC, 7 cases of type 2 PRCC), 12 cases of chrRCC (8 cases of common CRCC, CRCC C; 4 cases of CRCC accompanied with sarcomatous change, CRCC S), 9 cases of Xp11.2 RCC, 2 cases of undifferentiated RCC (unRCC). All original slides including hematoxylin-eosin and immunohistochemical staining from each case were reviewed and assessed in accordance with current diagnostic criteria by senior pathologists [9]. TNM stage was determined in all cases using 2009 staging criteria [10, 11] and followed up. Each paraffin block was reviewed to assure at least 70% tumor content before sectioning and DNA extraction.

DNA extraction

Total DNA was extracted from the samples using the standard phenol/chloroform extraction method, and peripheral blood cells were used as controls. DNA quality was checked on a 1% agarose gel and the amount of extracted DNA was measured spectrophotometrically at 260 nm (impurity and ratio of DNA to non-DNA were also cross-checked at 280 nm). Extractions were stored at -80°C prior to labeling by nick translation.

Comparative genomic hybridization

Comparative genomic hybridization was performed according to the manufacturer's protocol (Vysis Inc., U.S). Briefly, labeling reactions were performed with 1 μg DNA and the nick translation labeling kit (Vysis) in a volume of 50 μl with 0.1 mmol/L dNTP pool containing 0.3 mmol/L each dATP, dGTP, and dCTP; 0.1 mmol/L dTTP; 0.2 mmol/L fluorescein isothiocyanate (FITC)-dUTP (for the experimental sample) or cyanine 3 (Cy3)-dUTP (for the 46, XY male reference); nick translation buffer and nick translation enzyme. Probe size was determined by separation on 1% agarose gel. Metaphase slides were denatured at 73 ± 1°C for 5 min in 70% methanamide/2× SSC and dehydrated in an ethanol series (70%, 85%, and 100%). The hybridization mixture consisted of approximately 200 ng Spectrum Red total genomic reference DNA coprecipitated with 10 μg human Co-1 DNA (Invitrogen, USA) and dissolved in hybridization buffer before hybridization to metaphase chromosomes. The probe mixtures were denatured at 73°C for 5 min then competitively hybridized to the denatured normal metaphase chromosomes in a humid chamber at 37°C for 3 days. After washing, chromosomes were counterstained with 4,6-diamidino-2-phenylindole-2 HCl (DAPI II; Vysis) and embedded in an antifading agent to reduce photobleaching.

Microscopy and digital image analysis

A fluorescence microscope equipped with appropriate filters (DAPI, FITC, and Cy3) was used to visualize the signals. For each hybridization panel, raw images from at least 5 metaphases were captured through a computer-driven CCD camera and analyzed with ISIS image software (Carl Zeiss Inc., Germany). Chromosomes were indentified by their DAPI banding patterns. Threshold levels of 1.25 and 0.8 were used to score gains and losses, respectively. High-level amplification was indicated by a ratio

Table 1. Characteristics and follow-up data of 46 cases of RCC

No	Type	Gender/age	pTNM	Stage	Follow up	Survival time (years)
1	CCRCC	F/31	T2M0N0	2	survive	14
2	CCRCC	F/25	T1M0N0	1	Death	21
3	CCRCC	M/52	T2M0N0	2	Death	20
4	CCRCC	M/39	T2M0N0	2	Survive	18
5	CCRCC	M/56	T1M0N0	1	Survive	17
6	CCRCC	M/55	T3M0N0	3	Death	1
7	CCRCC	M/39	T3M0N0	3	Survive	10
8	CCRCC	F/74	T2M0N0	2	Survive	9
9	CCRCC	M/70	T2M0N0	2	Survival	8
10	CCRCC	M/70	T2M1NO	4	Death	16
11	Type 1 PRCC	M/62	T1M0N0	1	Survival	18
12	Type 1 PRCC	M/49	T2M0N0	2	Death	13
13	Type 1 PRCC	F/61	T2M0N0	2	Survival	13
14	Type 1 PRCC	M/36	T1M0N0	1	Survival	10
15	Type 1 PRCC	F/48	T2M0N0	2	Survival	6
16	Type 1 PRCC	M/52	T2M0N0	2	Survival	3
17	Type 2 PRCC	M/58	T3M0N0	3	Death	29
18	Type 2 PRCC	F/56	T2M0N0	2	Death	29
19	Type 2 PRCC	M/70	T3M0N0	3	Death	2
20	Type 2 PRCC	M/53	T2M0N0	2	Death	5
21	Type 2 PRCC	F/55	T2M0N1	3	Death	7
22	Type 2 PRCC	M/61	T2M1N0	4	Death	4
23	Type 2 PRCC	M/88	T2M1N0	4	Death	4
24	CRCC C	F/52	T2M0N0	2	Death	34
25	CRCC C	F/36	T1M0N0	1	Survival	19
26	CRCC C	M/42	T1M0N0	1	Survival	16
27	CRCC C	F/72	T3M0N0	3	Death	15
28	CRCC C	M/32	T1M0N0	1	Survival	13
29	CRCC C	F/74	T2M0N0	2	Survival	9
30	CRCC C	F/30	T1M0N1	3	Death	5
31	CRCC C	M/36	T2M0N0	2	Survival	7
32	CRCC S	M/25	T3M0N1	3	Death	34
33	CRCC S	F/32	T4M0N0	4	Death	13
34	CRCC S	M/75	T2M1NO	4	Death	11
35	CRCC S	M/64	T2M1N1	4	Death	10
36	Xp11.2RCC	M/26	T2M0N0	2	Death	37
37	Xp11.2RCC	M/47	T3M0N0	3	Death	36
38	Xp11.2RCC	F/47	T2M0N0	2	Death	26
39	Xp11.2RCC	M/85	T3M0N0	3	Survival	9
40	Xp11.2RCC	M/43	T1M0N1	4	Death	8
41	Xp11.2RCC	M/64	T1M0N0	1	Death	8
42	Xp11.2RCC	M/72	T2M1NI	4	Death	5
43	Xp11.2RCC	F/63	T2M1NO	4	Survival	4
44	Xp11.2RCC	F/72	T2M0N1	4	Survival	5
45	UnRCC	M/56	T3M0N0	3	Death	2
46	UnRCC	M/60	T3M1NO	3	Death	3

greater than 1.5. All centromeres, as well as chromosome p35-36 and the heterochromatic regions of chromosome 22, were excluded from further analysis, because these regions can yield unreliable hybridization data due to incompletely suppressed repetitive DNA sequences. Positive and negative controls provided comparisons for evaluation and interpretation of the data. Normal female DNA (labeled green) was used as negative control and normal male DNA was used for reference (labeled red). The intensity profiles for this experiment should be within the threshold values as determined by image analysis. DNA from MPE600 cells (with known genetic aberrations that are easy to detect by comparative genomic hybridization) was used as a positive control (labeled green) and normal male DNA was used as reference.

Statistical analysis

Pearson's χ^2 test or Fisher's exact test were used to compare differences between different groups. Multivariate cox regression analysis was used to analyze the risk factors of RCC prognosis. All data were analyzed using SPSS 17.0 statistical software. A *P*

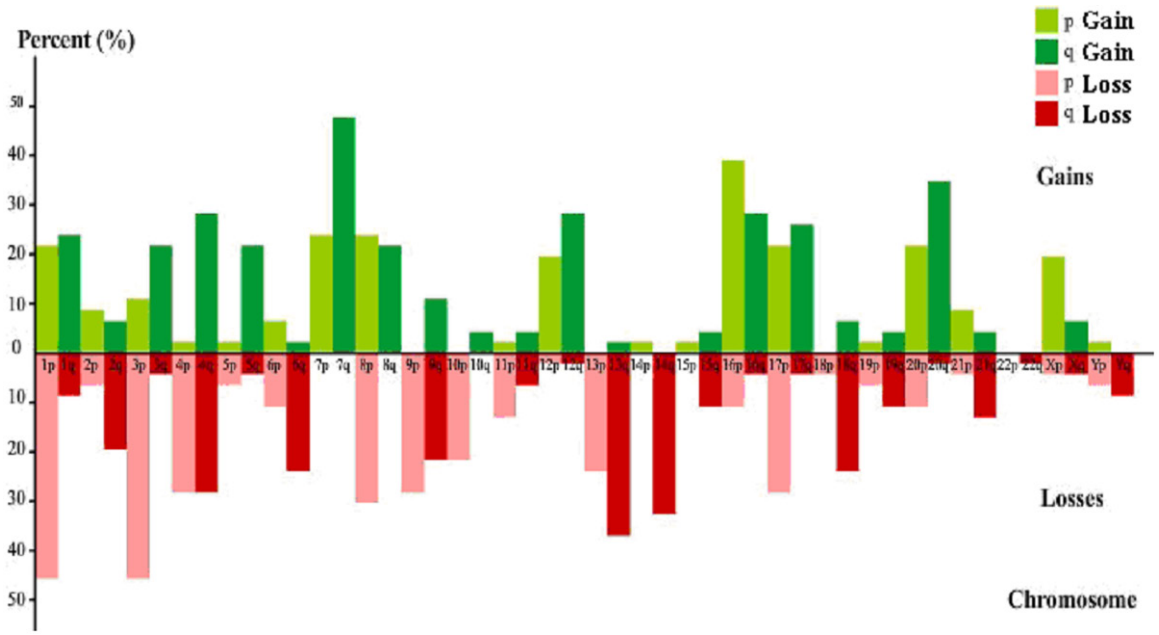

Figure 1. Frequency of chromosome gains and losses in renal cell carcinoma. Green histograms are gains, red are losses.

Table 2. Common large region of chromosome aberrations in renal cell carcinoma cases (>10%)

Chromosome number	Gain	Number (*n*=46)	RR	95% CI	*P*	Loss	Number (*n*=46)	RR	95% CI	*P*
1	1p11-13	8	0.850	0.328-2.206	0.738	1p11-22	19	1.170	0.529-2.589	0.698
	1p20-32	9	0.824	0.329-2.063	0.680	1p31-ter	13	0.700	0.259-1.887	0.480
3	3p11-ter	7	0.556	0.191-1.678	0.304	3p11-14	7	2.681	0.888-8.100	0.080
	3q13-29	8	1.019	0.366-2.841	0.971	3p20-ter	15	0.720	0.245-2.121	0.552
4	4q21-32	6	0.466	0.130-1.665	0.240	4p12-ter	9	1.478	0.508-4.300	0.473
						4q	14	2.360	0.854-6.519	0.098
5	5q21-ter	9	1.830	0.6.9-5.497	0.561					
6						6q	13	1.389	0.469-4.111	0.553
7	7p13-ter	9	0.350	0.109-1.121	0.077					
	7q11	7	1.757	0.605-5.103	0.300					
	7q21-22	18	1.950	0.743-5.118	0.175					
	7q31-ter	16	1.680	0.517-5.457	0.388					
8	8p12-ter	9	1.575	0.546-4.544	0.401	8p-	12	0.529	0.209-1.340	0.179
	8q20-ter	10	1.392	0.562-3.448	0.475					
9	9q12-31	5	0.532	0.130-2.181	0.380	9p	10	0.796	0.282-2.252	0.668
						9q12-33	13	0.851	0.345-2.094	0.725
10						10p12-ter	10	1.665	0.603-4.598	0.325
12	12p11-ter	7	3.502	1.097-11.182	0.034					
	12q14-ter	14	5.115	1.847-14.170	0.002					
13						13p-	12	1.137	0.418-3.097	0.801
						13q12-22	19	1.992	0.761-5.214	0.160
14						14q-	14	1.442	0.576-3.612	0.434
16	16p12-13	7	0.557	0.148-2.100	0.387					
	16q21-24	11	2.629	1.027-6.731	0.044					
17	17p12-ter	6	3.643	1.262-10.512	0.017	17p12-ter	11	1.221	0.378-3.942	0.739
	17q12-ter	10	1.330	0.464-3.810	0.595					
18						18q12-23	11	2.911	1.006-8.425	0.049
19						19q-	6	1.842	0.540-6.282	0.329
20	20p	8	1.141	0.241-5.404	0.868					
	20q12-ter	17	1.044	0.446-2.444	0.920					

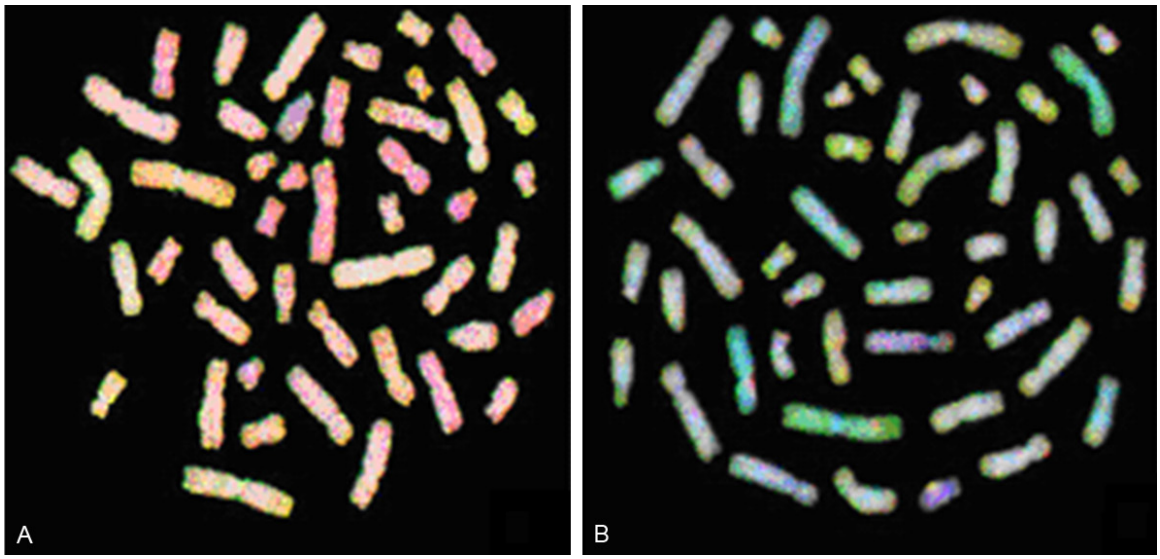

Figure 2. Comparative genomic hybridization metaphase spreads of RCC 20 (A) and RCC 25 (B). Green areas are gains, red areas are losses, yellow/yellowish areas are normal, and blue areas are heterochromatin. Hybridization to repetitive sequences/heterochromatin were blocked by unlabeled human Cot-1 DNA and stained blue with 4,6-diamidino-2 phenylindole-2 HCL (DAPI).

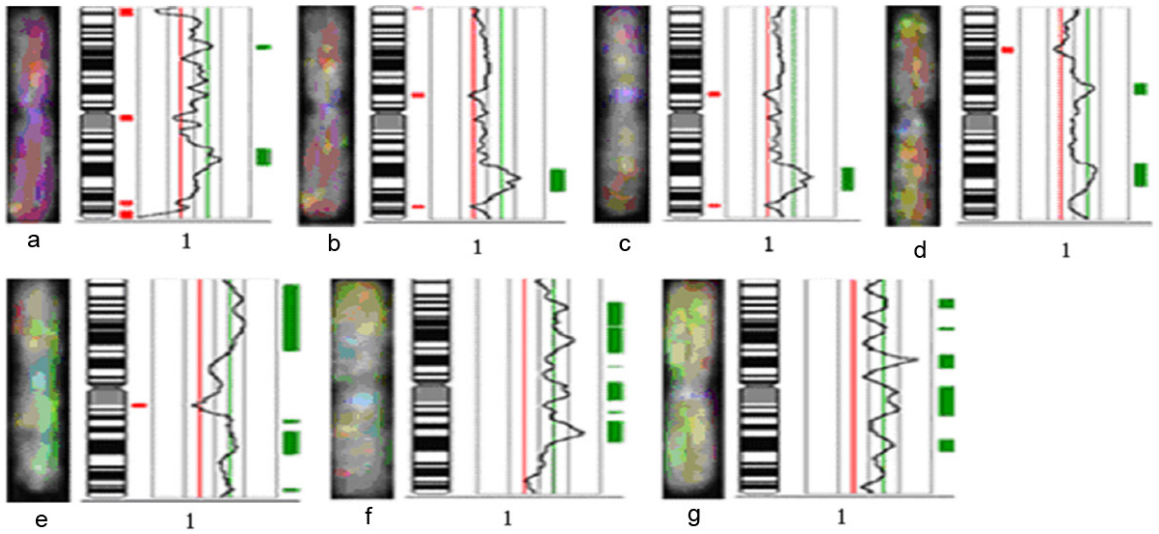

Figure 3. Comparative genomic hybridization profiles of chromosome 1. Green to red fluorescence thresholds (represented by the green/red line) are 0.8 and 1.25, respectively. The curve shows DNA copy status. Curves to the left of the red line indicate losses, curves to the right indicate gains. a, b, c, d, e, f, g represent RCC cases 3, 20, 32, 4, 24, 25, and 27, respectively.

value < 0.05 was considered statistically significant.

Results

Subject characteristics

A total of 46 RCC tumors were included in the analysis. **Table 1** lists host and tumor characteristics. The mean age was 53.6 years. Men were overrepresented in the group (1.87:1). Using the 2009 TNM classification for renal cell carcinoma [10, 11], 8 patients had stage I, 15 had stage II, 13 had stage III, and 10 had stage IV tumors (17.4%, 32.6%, 28.3%, and 21.7%).

Comparative genomic hybridization profiles

Comparative genomic hybridization revealed DNA sequence gains and losses in all 46 pri-

Table 3. Characteristic chromosomal change in different subtype of renal cell carcinoma

	N	Characteristic chromosomal change									
		-3p21-25	*P*	-15q	*P*	+16p11-13	*P*				
CCRCC	10	8		4		6					
*Non-CCRCC	34	5	*0.0003*	1	*0.0130*	1	*0.0001*				
**PRCC	13	5	*0.0903*	1	*0.1269*	1	*0.0186*				
**CRCC	12	0	*0.0001*	0	*0.0287*	0	*0.0281*				
**Xp11.2RCC	9	0	*0.0007*	0	*0.0867*	0	0.0108				
		+7p13-22	*P*	+8q21-24	*P*	-18q12-ter	*P*	-14q13-24	*P*	-Xp11-q13/-Y	*P*
PRCC	13	8		7		8		8		7	
1 type PRCC	6	4		1		5		4		4	
**2 type PRCC	7	4		6	*0.0291*	3		4		3	
*Non-PRCC	31	2	*0.0003*	4	*0.0131*	2	*0.0003*	4	*0.0033*	0	*0.0001*
**CCRCC	10	0	*0.0059*	0	*0.0003*	1	*0.0288*	*0*	*0.0059*	0	*0.0075*
**CRCC	12	0	*0.0016*	0	*0.0001*	0	*0.0016*	*0*	*0.0016*	0	*0.0052*
**Xp11.2RCC	9	2	*0.0990*	4	*0.1312*	1	*0.0306*	4	*0.6656*	0	*0.0167*
		-2q12-32	*P*	^3p+	*P*	^^-10p12-15	*P*	-11p11-15	*P*	^^-13p11-ter	*P*
CRCC	12	6		4		7		5		7	
CRCC C	8	4		0		7		3		7	
**CRCC S	4	2		4	*0.0020*	0	*0.0101*	2		0	*0.0101*
*Non-CRCC	32	2	*0.0036*	0	*0.0046*	3	*0.0023*	0	*0.0008*	4	0.0062
**CCRCC	10	1	*0.0743*	0	*0.0010*	0	*0.0003*	0	*0.0396*	0	*0.0003*
**PRCC	13	0	*0.0052*	0	*0.0004*	1	*0.0005*	0	*0.0372*	1	*0.0005*
**Xp11.2RCC	9	1	*0.1588*	0	*0.0014*	2	*0.0152*	0	*0.0451*	3	*0.0497*
		+Xp11-12	*P*	+12q12-24	*P*	-14q13-24	*P*				
Xp11.2RCC	9	6		6		4					
*Non-Xp11.2RCC	35	0	*0.0000*	6	*0.1946*	*8*	*0.3803*				
**CCRCC	10	0	*0.0030*	1	*0.0572*	0	*0.0325*				
**PRCC	13	0	*0.0011*	5	*0.6656*	8	*1.0*				
**CRCC	12	0	*0.0015*	0	*0.0062*	*0*	*0.0210*				

*Pearson χ^2 test; **Fisher exact test; +: gain; -: lose; ^*P* value from the comparison between CRCC S group and other type RCC; ^^*P* value from the comparison between common CRCC group and other type RCC.

mary renal cell carcinoma cases, with 260 gains and 282 losses. The mean numbers of aberrations per tumor sample were 5.7 gains and 6.1 losses. Gains were most frequently detected on chromosomes 7q, 16p, and 20q, common large regions of chromosome gains in RCC cases were most frequently detected on chromosomes 7q21-22, 7q31-ter, 12q14-ter, 16q21-24, and 20q12-ter. Frequently occurring losses involved 1p, 3p, 13q, 14q, and 8p, involving 1p31-ter, 3p20-ter, 4q, 6q, 8p, 9q12-33, 13q12-22, and 14q (**Figure 1**; **Table 2**).

Chromosomal changes in different renal cell carcinoma subtypes

Comparative genomic hybridization profiles showed that chromosomal changes varied among the 4 renal cell carcinoma subtypes (10 CCRCC, 13 PRCC, 12CRCC, 9 Xp11.2RCC). Representative analyses are shown in **Figures 2** and **3**. There are relative characteristic chromosomal changes in different subtypes of RCC (**Table 3**).

In CCRCC, the most frequently occurring chromosomal gains and losses were 7q (9/10), 16p (8/10), 5q (6/10), and 3p (9/10), 8p (8/10), 1p (7/10), 4p, 4q, 9p (6/10), 9q, and 14q (3/10). The gain of 16p11-13 is more frequent in CCRCC than in other types of RCC (Non-CCRCC, P = 0.001, PRCC, P = 0.0186, CRCC, P = 0.0281, Xp11.2RCC, P = 0.0108); the loss of 3p21-25 is more frequent in CCRCC than in chrRCC (P = 0.0001) and Xp11.2RCC (P = 0.0007); the loss of 15q is more frequent in CCRCC than in CRCC (P = 0.0287).

In PRCC, gains were seen in chromosome arms 7p, 7q, 12q (8/13), 16q, 20p, 20q (7/13), 8q, 16p, 17q (6/13), 12p, 17p (5/13), and 8p (4/13), and losses occurred frequently on chromosome 14q, 18q (8/13), 13q (7/13), 3p, 4p, 6q (6/13), 1p, 4q, 9p (5/13), Yq (4/13), and Xp (3/13). The gain of 8q21-24 was more apparent in type 2 PRCC than in type 1 PRCC (P = 0.0291), CCRCC (P = 0.0003), and CRCC (P = 0.0001); the loss of 18q12-ter and Xp11-q13/Y is more frequent than in other types of RCC (-18q12-ter: Non-PRCC, P = 0.003, CCRCC, P = 0.0288, CRCC, P = 0.0016, Xp11.2RCC, P = 0.0306, -Xp11-q13/Y: Non-PRCC, P = 0.001, CCRCC, P = 0.0075, CRCC, P = 0.0052, Xp11.2RCC, P = 0.0167), the gain of 7q13-22 and loss of 14q13-24 is relative more apparent in PRCC contrasted with CCRCC (+7q13-22: P = 0.0059, -14q13-24, P = 0.0059), and CRCC (+7q13-22: P = 0.0016, -14q13-24, P = 0.0016).

For CRCC, gains were seen in chromosome arms 1q (7/12), 3q (6/12), 1p (5/12), and 3p, 4q, 9q, 16p (4/12), and losses occurred frequently on chromosome 1p, 17p (8/12), 10p, 13p (7/12), 2q, 8p (6/12), 11p, 21q (5/12), and 6q, 13q (4/12). The gain of 3p, loss of 11p11-15, and 13p11-ter significantly differ between common CRCC and groups associated with sarcomatous change (P = 0.0020, P = 0.0101, P = 0.0101). The gain of 3p is more frequent in CRCC accompanied by sarcomatous change than in other types of RCC (CCRCC, P = 0.0010, PRCC, P = 0.0004, Xp11.2RCC, P = 0.0014), loss of 10p12-15, and 13p11-ter is more frequent in common CRCC than in other types of RCC (-10p12-15: CCRCC, P = 0.0003, PRCC, P = 0.0005, Xp11.2RCC, P = 0.0152; 13p11-ter: CCRCC, P = 0.0003, PRCC, P = 0.0005, Xp11.2RCC, P = 0.0497). The loss of 11p11-15 is more frequent in CRCC than in other types of RCC (CCRCC, P = 0.0396, PRCC, P = 0.0372, Xp11.2RCC, P = 0.0152), and the frequency of loss of 2q12-32 significantly differs between CRCC and PRCC (P = 0.0052).

In Xp11.2RCC, gains were seen in chromosome arms Xp (6/9), 7q, 12q (5/9), 8p, 8q, 16q, 17p, 17q, 20q (4/9), and 5q, 7p, 12p (3/9), and losses occurred frequently on chromosome 3p, 9q, 14q (4/9), and 16p (3/9). Gain of Xp11-12 is more frequent in Xp11.2RCC than in other types of RCC (CCRCC, P = 0.0030, PRCC, P = 0.0011, CRCC, P = 0.0015); loss of 14q13-24 is more frequent than in CCRCC (P = 0.0325) and CRCC (P = 0.0210); gain of 12q12-24 differs between Xp11.2RCC and CRCC (P = 0.0062).

Comparison of chromosomal changes with clinicopathological parameters

Follow-up data revealed the prognosis of RCC is associated with clinicopathologic stage (P = 0.004) and patient age (P = 0.002). The mortality risk of stage II RCC is 1.684 (0.341-8.311) vs. stage I RCC but the difference is not significant (P = 0.523). In comparison to stage I RCC, the mortality risk of stage III and IV RCC are 5.119 (95% CI: 1.052-25.679; P = 0.043), 11.187 (2.173-57.597; P = 0.004), respectively. Advanced age is associated with increased

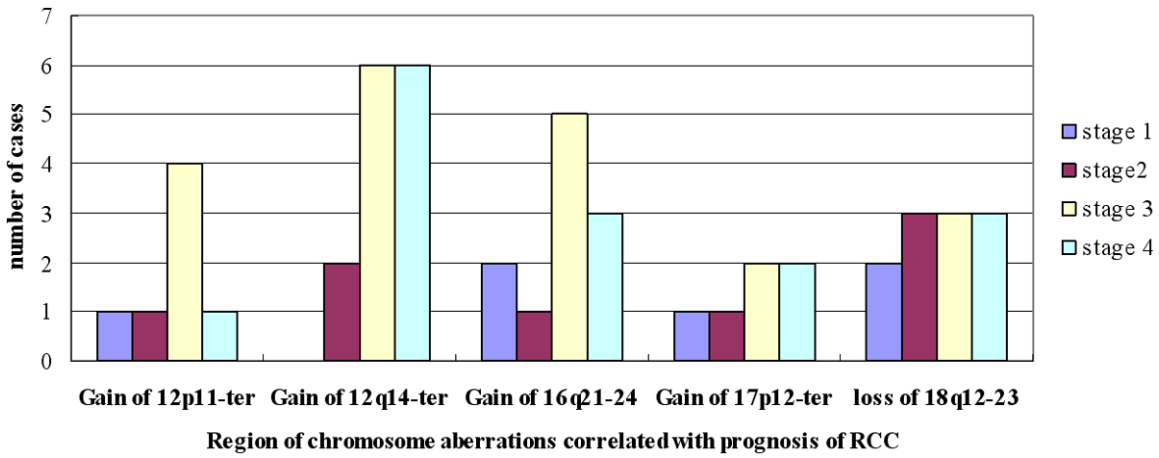

Figure 4. Stage distribution of the cases harboring 2p11-ter, 12q14-ter, 16q21-24, 17p12-ter, 18q12-23 aberrations.

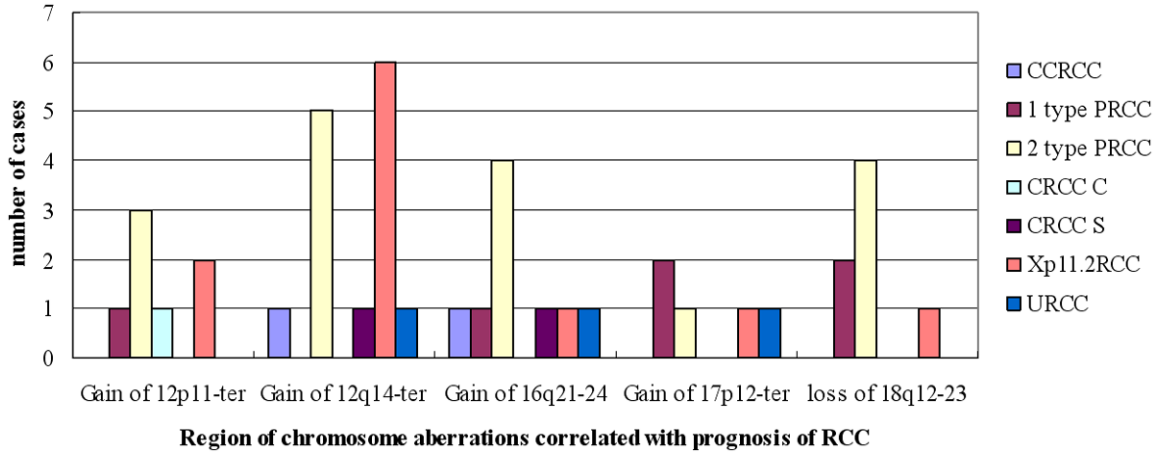

Figure 5. Type distribution of the cases harboring 2p11-ter, 12q14-ter, 16q21-24, 17p12-ter, 18q12-23 aberrations.

mortality risk. After controlling for clinicopathologic stage, age and gender, the multivariate Cox regression analysis showed the gain of chromosome 12p11-ter (*P* = 0.034, RR = 3.502, 95% CI 1.097-11.182), 12q14-ter (*P* = 0.002, RR = 5.115, 95% CI 1.847-14.170), 16q21-24 (P = 0.044, RR = 2.629, 95% CI 1.027-6.731), 17p12-ter (*P* = 0.017, RR = 3.643, 95% CI 1.262-10.512) and the loss of 18q12-23 (*P* = 0.049, RR = 2.911, 95% CI 1.006-8.425) is correlated with prognosis of RCC (**Table 2**). The type and stage distribution of the cases harboring 2p11-ter, 12q14-ter, 16q21-24, 17p12-ter, 18q12-23 aberrations were show in **Figures 4** and **5**, which correlated with poorer prognosis. The regions were more common in Xp11.2RCC, 2 type PRCC and CRCC associated with sarcomatous change vs. other subtype of RCC, and more frequent in stage III-IV than stage I-II.

Discussion

Renal cell carcinoma is a group of malignancies arising from the epithelium of the renal tubules where histological classification of tumor subtypes is sometimes equivocal. Comparative genomic hybridization is a convenient and rapid way to screen for chromosomal changes. There have been some studies of renal cell carcinoma by CGH, but most focus on CCRCC and PRCC [12-16]. This study is the first attempt to use this method in differential diagnosis of 4 main subtypes RCC, and advances our understanding of the molecular basis of renal cell carcinoma, which may provide clues to new genes involved RCC tumorigenesis.

In this preliminary study, we performed genome-wide screening to detect genetic changes associated with clinical parameters in primary renal

cell carcinoma. We detected DNA gains and losses in all 46 cases investigated; losses were more common than gains. Losses (in order of frequency) were detected at chromosomes 1, 3, 13, 14, and 8. For CCRCC, there were consistent losses of whole or partial arms of several chromosomes, notably chromosomes 3, 8, 1, 4, 9, and 14. The chromosomes with consistent losses in PRCC were 14, 18, 13, 3, 4, 6, 1, 9, and Y. In CRCC, chromosomes 1, 17, 10, 13, 2, 8, 11, 21, and 6 consistently exhibited losses. Chromosomes 3, 9, 14, and 16 sustained consistent losses in Xp11.2RCC. For unRCC, the chromosomes sustaining chromosomal losses were 1, 2, 3, 5, 6, 11, 16, 17, and 20. Gains were infrequent in the 5 subtypes. These results are consistent with previous reports [15, 17-19].

Our study shows the CGH assay is a useful complementary method for differential diagnosis of the 4 main subtypes of RCC. The comparisons of chromosome aberrations in CCRCC, PRCC, CRCC, and Xp11.2 RCC revealed characteristic differences. These results are mainly consistent with previous reports [14, 15, 20-22], but we also identified new regions that are helpful in differential diagnosis of RCC. For CCRCC, in addition to loss of 3p21-25, the loss of 15q and gain of 16p11-13 are also relatively frequent in comparison to other subtypes of RCC. In PRCC, gain of 7p13-22 and 8q21-24 and loss of 18q12-ter, 14q13-24, and Xp11-q13/Y are more frequent than in other types of RCC, and gain of 8q21-24 is more characteristic of type 2 than type 1 PRCC. For CRCC, the loss of 2q12-32, 10p12-15, 11p11-15, and 13p is helpful in differential diagnosis with other types of RCC, and the gain of 3p and loss of 11p11-15 and 13p significantly differ between common CRCC and CRCC accompanied by sarcomatous change. Gain of Xp11-12 is more frequent in Xp11.2RCC than in other types of RCC.

Based on Cox regression analysis, 5 chromosome region aberrations were poor prognostic indicators in RCC, including the gain of chromosome 12p12-ter, 12q14-ter, 16q21-24, and 17p12-ter and loss of 18q12-23, which may provide clues to new genes involved in RCC tumorigenesis.

The first region 12p11-ter was associated with RCC in Cox analysis ($P = 0.034$, RR = 3.502, 95% CI 1.097-11.182). This region, which contains the P27 gene, occurs frequently and is a strong predictor of poor survival in RCC [23-26]. Recent research has shown that p27 is phosphorylated at T157 of the NLS, causing inhibition of phosphatidylinositol 3-kinase (PI3K), reducing AKT activity and T157 phosphorylation and inducing nuclear relocalization of p27. Clinical testing of these findings may provide a rational method for use of PI3K/AKT pathway inhibitors in patients with RCC [25].

Our finding on 12q14-ter is notable because the small locus on 12q24.31 for rs4765623, which maps to SR-B1, the scavenger receptor class B, member 1 gene, has recently been implicated in RCC [27]. The SCARB1 gene encodes a cell-surface receptor that binds to high-density lipoprotein cholesterol (HDL-C) and mediates HDL-C uptake. Polymorphisms in the SR-BI gene (SCARB1) are associated with variations in plasma lipoprotein profile and other risk factors for cardiovascular disease [28-31]. Its role in cancer biology is not well established, as the proportion of all cases of RCC attributable to excess weight and obesity has been estimated to be about 40% in the United States and up to about 30% in European countries [5, 32, 33]. The mechanisms by which obesity influences renal carcinogenesis are not clear. Further investigation is required to study its association with RCC risk.

The third region 16q21-24 may harbor a tumor suppressor gene that controls cell proliferation and loss of function leads to a growth advantage and transformation of low-grade to high-grade tumors. The identity of this gene or genes remains unknown. One interesting note is that E-cadherin is located on 16q21-24, which modulates cell adhesion and cell polarity. The repression of E-cadherin in renal tubular cells may participate in the events of epithelial to mesenchymal transition (EMT) that plays an important role in progressive kidney disease [34], and the E-cadherin repressor Snail is associated with cancer invasion and prognosis [35].

The genes on 17p12-ter remain unclear, but one interesting gene is TP53, a well-known tumor suppressor gene. There is some data on the relationship between TP53 and RCC, although the results are controversial. Some researchers believe RCC patients with tumors

expressing increased p53 and MDM2 may have the poorest overall survival [36]. TP53, Profilin 1 may be a key element in the pathological processes of RCC; it has the potential to serve as a diagnostic or progression biomarker and therapeutic target in RCC [37]. Other results suggest p53 expression may not play a role in prognosis of RCC [38].

The loss of 18q12-23 is significant because the small locus on 18q21.3 has been implicated in RCC [39], and recent research suggests inactivation of genes at 18q12-23, including SMAD 2/4/7, Smad-ubiquitination regulatory factor 2 (Smurf2), TGFBI, TCF-4, receptor activator of NF-kappaB ligand (RANKL) gene, may be involved in the tumorigenesis of RCC. For example, immunoreactivity to nuclear phosphorylated Smad2 was significantly lower in RCC than in normal renal tissues [40]. The level of Smurf2 was greater in RCC tissues of patients with advanced clinical stages vs. normal tissues [40]. TGFBI can promote metastasis of RCC cells depending on inactivation of the VHL tumor suppressor; TGFBI could be a therapeutic target against RCC in the future [41]. The imbalance between TCF-4 gene splicing isoforms with long and short reading frames is associated with RCC progression through inhibition of the apoptotic pathway [42]. RANKL and its receptor, receptor activator of NF-kappaB (RANK) was observed in metastatic RCC in the bone and other organs, suggesting they play a role in metastasis to the bone and other organs. Multivariate Cox analysis revealed that the RANKL-RANK-OPG system is involved not only in bone metastasis of RCCs but also in metastasis to other organs through the stimulation of cancer cell migration [43].

In summary, comparative genomic hybridization analysis revealed novel genomic imbalances in primary renal cell carcinoma. The results of this study suggest comparative genomic hybridization is a useful complementary method for differential diagnosis of RCC and for detecting alterations in large, critical chromosomal regions in renal cell carcinoma. Five chromosome regions with aberrations achieved bad prognosis significance of RCC, including the gain of chromosome 12p11-ter, 12q14-ter, 16q21-24, and 17p12-ter and the loss of 18q12-23, which may provide clues to new genes involved in RCC tumorigenesis. Further analysis to map genes to specific regions is underway to determine the contributions of these genes to the development of renal cell carcinoma.

Acknowledgements

Supported by grants from the National Natural Science Foundation of China (NSFC, No. 81060209) and from the International S&T Cooperation Program of China (2010DFB34-100).

Disclosure of conflict of interest

None.

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