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Genomic imbalances in 61 renal cancers from the proximal tubulus detected by comparative genomic hybridization

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Abstract. Comparative genomic hybridization (CGH) has been applied to characterize 61 primary renal cell carcinomas derived histogenetically from the proximal tubulus. The tumor samples comprised 46 clear-cell renal cell carcinomas (ccRCCs) and 15 papillary renal cell carcinomas (pRCCs). Changes in the copy number of entire chromosomes or subregions were detected in 56 tumors (92%). In ccRCCs, losses of chromosome 3 or 3p (63%); 14q (30%); 9 (26%); 1 and 6 or 6q (17% each); 4 and 8 or 8p (15% each); 22 (11%); 2 or 2q and 19 (9% each); 7q, 10, 16, 17p, 18, and Y (7% each); and 5, 11, 13, 15, and 21 (4% each) were detected. Most frequent genomic gains in ccRCC were found on chromosome 5 (63%); 7 (35%); 1 or 1q (33%); 2q (24%); 8 or 8q, 12, and 20 (20% each); 3q (17%); 16 (15%); 19 (13%); 6 and 17 or 17q (11% each); and 4, 10, 11, 21, and Y (9% each). In pRCCs, gains in the copy number of chromosomes 7 and 17 (7/15, each) and 16 and 20 (6/15, each) were frequent. One pRCC showed amplification of subchromosome regions 2q22→q33, 16q, 17q and the entire X chromosome. In pRCC, losses were less frequently seen than gains. Losses of chromosomes 1, 14, 15, and Y (3/15 each) and 2, 4, 6, and 13 (2/15 each) were observed. In ccRCCs, statistical evaluation revealed significant correlations of chromosomal imbalances with tumor stage and grade, i.e., a gain in copy number of chromosome 5 correlated positively with low tumor grade, whereas a gain of chromosomes 10 and 17 correlated positively with high tumor grade. Furthermore, loss of chromosome 4 correlated positively with high tumor stage.

In past decades the incidence of renal cancer has increased such that, today, 3% of all human neoplasias are renal cancers. In Germany, approximately 10,000 patients will be affected by renal cancer annually, and almost half of them will die from the disease. Renal cell carcinomas have been shown to be very heterogenous with respect to clinical picture, histology, and genetic properties (Presti et al., 1993; van den Berg et al., 1993). Recently, a pathological classification system of renal cancer, first proposed in 1986 (Thoenes et al., 1986), has been updated (Kovacs et al., 1997; Störkel et al., 1997). Today, subgroups of renal cell tumors are distinguished based on morphological, immunohistochemical, and genetic criteria (van den Berg et al., 1993; Störkel and van den Berg, 1995).

This classification system defines five major subgroups based on histogenetic origin. Clear-cell renal cell carcinoma (ccRCC) accounts for approximately 70% of all renal tumors. Papillary renal cell carcinoma (pRCC), with an incidence of 10% to 15%, represents the second most common subtype. Chromophobe carcinoma (5% to 10%), renal oncocytoma (a benign tumor with an incidence of 5% to 7%), and the rare, highly aggressive collecting duct carcinoma account for the rest.

Cytogenetic studies (Kovacs et al., 1987, 1988; Kovacs and Frisch, 1989; Meloni et al., 1992; Hughson et al., 1993) and allelotyping studies applying restriction fragment length polymorphism and microsatellite techniques (Anglard et al., 1991;

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Morita et al., 1991; Ogawa et al., 1992; Presti et al., 1993; Foster et al., 1994; Thrash Bingham et al., 1995a, b; van den Berg et al., 1996; Schullerus et al., 1997; van den Berg and Buys, 1997; Hughson et al., 1998) have provided the genetic basis for the classification system. In the recent past, these techniques have been extended by complementary molecular cytogenetic techniques, such as multicolor fluorescence in situ hybridization (M-FISH) and comparative genomic hybridization (CGH) (Kallioniemi et al., 1993; du Manoir et al., 1993; Kallioniemi et al., 1994; Piper et al., 1995). CGH overcomes some specific technical limitations of both conventional cytogenetics and allelotyping and is not dependent on the mitotic activity of the tumor cells, as is cytogenetics. Since no cell culture is required, the actual status of the neoplasia is captured directly, and the results are representative of the primary tumor. Compared to allelotyping, CGH gives an overview over the complete genome of the tumor cells. The disadvantages of CGH, such as the inability to detect balanced translocations, its low resolution, and lack of a single-cell approach are well known. Therefore, CGH is an add-on technique to cytogenetic and molecular genetic approaches.

In the present study, 61 primary renal cell carcinomas (46 ccRCCs and 15 pRCCs) were analyzed by CGH. The objectives of our study were twofold: (1) to correlate subtype-specific genetic imbalances with clinicopathological parameters, such as nuclear grading, pathological stage, tumor size, gender, patients' age at surgery, and survival, and (2) to search for additional subtype-specific genomic alterations.

Table 1. Genomic imbalances observed in 46 cases of clear-cell renal cell carcinoma (ccRCC) and 15 cases of papillary renal cell carcinoma (pRCC)[a]

Chromosome No.	Losses in copy number (%)		Gains in copy number (%)	
	ccRCC	pRCC	ccRCC	pRCC
1	**17**	**20**	**33**	**20**
2	9	13	**24**	13
3	**63**	7	**17**	**27**
4	**15**	13	9	13
5	4	7	**63**	**20**
6	**17**	13	11	0
7	7	7	**35**	**47**
8	**15**	0	**20**	7
9	**26**	0	7	13
10	7	0	9	7
11	4	7	9	7
12	2	0	**20**	**33**
13	4	13	7	13
14	**30**	**20**	0	7
15	4	**20**	4	7
16	7	0	**15**	**40**
17	7	7	11	**47**
18	7	7	4	0
19	9	7	13	13
20	0	0	**20**	**40**
21	4	7	9	7
22	11	0	4	7
X	0	0	9	7
Y	7	**20**	4	0

[a] Values shown in boldface are the most frequent alterations (≥ 15% of cases).

Materials and methods

Materials

Primary tumor specimens of 61 RCCs from one center, the University Hospital of Mainz, were studied. Samples were collected randomly following radical nephrectomy over a period of 9 yr (1990–1999). Tumor tissues were snap-frozen in liquid nitrogen immediately after surgery and stored at −80 °C until DNA preparation. The tumors were from 39 men and 23 women. Patient age ranged from 29 to 90 yr, with a median age of 63 yr. The tumor's maximum diameter varied between 2.5 and 15 cm. Median diameter was 6.7 cm. TNM staging, grading, and histopathological evaluation were performed at the Institute of Pathology, University of Mainz, according to established criteria (Fuhrman et al., 1982; Kovacs et al., 1997; Medeiros et al., 1997; Störkel et al., 1997). Tumors classified prior to 1997 were re-evaluated with respect to the new TNM staging system (Guinan et al., 1997). A total of 31 tumors were stage I, 6 were stage II, 21 were stage III, and 3 were stage IV. There were 7 grade 1, 35 grade 2, and 18 grade 3 tumors. One tumor was grade 4. Clinical follow-up data were available from 38 patients. Informed consent was received from each patient prior to surgery. Median age at surgery was 62 and 58 yr in ccRCC and pRCC, respectively.

Chromosome preparations

Metaphase chromosome spreads were prepared from short-term cultured, phytohemagglutinin-stimulated lymphocytes of a healthy male donor. Chromosome preparations were performed applying standard cytogenetic procedures. Hypotonic treatment was performed for 15–20 min with 75 mM KCl. All preparations were fixed in cold (−20 °C) methanol:acetic acid (3:1).

DNA preparation

Reference DNA was isolated using a Roche Diagnostics DNA Isolation Kit for Blood. Test DNA was extracted from tumor samples using a High Pure PCR Template Preparation Kit (Roche Diagnostics). Preparations were performed following the manufacturer's recommendations. DNA was adjusted to a concentration of 1 μg/μl.

DNA labeling

Whole genomic DNA from peripheral normal blood (reference) and genomic tumor DNA (test) were labeled differentially with digoxigenin-11-dUTP and biotin-16-dUTP (Roche Diagnostics), respectively. Labeling was done by a standard nick-translation procedure (CGH Nick Translation Kit, Vysis). The length of labeled DNA ranged from 200 to 1,000 bp (according to tests after DNA denaturation on a 1 % agarose gel).

Comparative genomic hybridization (CGH)

CGH was performed according to du Manoir et al. (1993). Labeled reference and test DNA (500 ng each) were co-precipitated with 50 μg human Cot-1 DNA (GIBCO BRL) and resuspended in 12 μl of hybridization mixture (50 % formamide, 10 % dextran sulfate, and 2 × SSC [pH 7.0]). Metaphase chromosomes were denatured in 70 % formamide, 2 × SSC (pH 7.0) for 2 min at 72 °C. Probe denaturation was performed for 6 min at 72 °C. After pre-annealing of 30 min at 37 °C, the probe was applied to the denatured metaphase chromosomes. Hybridization was carried out for 3 d at 37 °C in a dark, humid chamber. Post-hybridization washes were done for 3 × 5 min in 50 % formamide, 2 × SSC at 42 °C with agitation, followed by high-stringency wash for 3 × 5 min with 0.1 × SSC at 60 °C without rocking. Unspecific binding was blocked by applying 3 % BSA in 4 × SSC, 0.1 % Tween 20 (Sigma). Fluorescein-isothiocyanate (FITC) conjugated to avidin and anti-digoxigenin-FAB conjugated to tetraethylrhodamine (TRITC) fragments (Q'Biogene) were used to detect the biotin- and digoxigenin-labeled probes, respectively. Post-detection washes were performed for 3 × 5 min with 4 × SSC, 0.1 % Tween 20 at 42 °C with agitation.

A Leica DMRBE epifluorescence microscope and a gray-level CCD camera were used. Image capturing and processing were performed using *Cytovision* version 3.1 (Applied Imaging) on a Dell Power Edge SP590-2 computer. At least 15 adequate metaphase spreads were captured for each case. A loss in copy number was recorded when the tumor-to-normal fluorescence ratio was <0.75, whereas a gain in copy number was recorded when the ratio was >1.25 (du Manoir et al., 1995). Chromosome gains exceeding a tumor-to-normal ratio of 1.5 were considered as copy number amplifications. CGH profiles of insufficient quality were excluded from evaluation.

Table 2. Chromosome imbalances (ISCN [1995] nomenclature) and clinicopathological data on 46 cases of clear-cell renal cell carcinoma

Case	Copy number gain	Copy number loss	Age at surgery (yr)	Tumor diameter (cm)	Grade	T	N	M	Gender
c1	rev ish enh(1q,2,5q21q35,7,8p21q24.3,12,20)	rev ish dim(3,9,14)	60	8.5	2	1	0	x	Female
c2	rev ish enh(2,5,18)	rev ish dim(1p36.3p31,3p,4,8,9,10q23q26,14,22)	55	12	2	3b	x	x	Female
c3	rev ish enh(1q,X)	rev ish dim(3p,6q21q27,16q)	75	5.5	3	1	x	x	Female
c4	rev ish enh(5p,7,12,17q22q25)	rev ish dim(3p,4q24q35,13q11q31,14,17p)	70	7	3	3b	x	x	Male
c5	rev ish enh(4q22q28,5q,12q11q22,16p12q24.3,20,21)	No imbalance detected	66	10	2	3a	x	x	Male
c6	rev ish enh(4q26q28,7,21)	rev ish dim(3p36q13.3,5q21q23,6q24q27)	62	4.5	1	1	x	x	Male
c7	rev ish enh(1q,3q,5q21q23,8q)	No imbalance detected	74	5.5	3	3a	x	x	Male
c8	rev ish enh(2q,5)	rev ish dim(3p,7q32q36,9)	83	5	3	1	x	x	Female
c9	rev ish enh(3q24q26.3,5p22q13,6q11q22,13q22q31)	rev ish dim(1p36.3p32,1q22,1q41q44,2p25p23,9p, 16p,18p11.3,19q)	55	5	1	1	x	x	Female
c10	No imbalance detected	rev ish dim(3p,9q,10q,11q,12q,14q,22q13)	58	6.5	3	3a	1	x	Male
c11	rev ish enh(2q24q34)	No imbalance detected	62	3	2	1	x	x	Male
c12	rev ish enh(1q,2q24q34,5q23q35,7,8q21.3q22)	rev ish dim(6q)	45	7	2	1	x	x	Male
c13	rev ish enh(1q,5q,7,8q,11,12,19,20,21)	rev ish dim(1p33p11,3,8p)	75	6.5	2	1	x	x	Male
c14	rev ish enh(5,7,20)	No imbalance detected	59	4	2	1	x	x	Male
c15	No imbalance detected	No imbalance detected	70	11	2	3b	0	x	Male
c16	rev ish enh(5,7,16)	rev ish dim(8,14)	50	7	2	1	x	x	Male
c17	rev ish enh(1q,2,3q,5q13q35,5p15.2p14,6,7p22q31, 8q22q24.1,10q,11,12q13q24.1,13q21.3q31,15q,17q21, 18q11.2q21)	rev ish dim(1p36.3p32,3p26p14,4,7q34q36,8p,9, 14q,21q)	65	12.5	2	2	x	x	Male
c18	rev ish enh(1p34.2q44,5,12p)	rev ish dim(2q34q37,3p26q24,4,14)	62	6.3	2	2	x	1	Male
c19	rev ish enh(Xq22q28)	rev ish dim(3p26p14)	44	11	2	3b	x	x	Male
c20	rev ish enh(1p36.3p33,5q23q35,7,19,20q,21,22)	rev ish dim(3p,Y)	57	5	2	1	x	x	Male
c21	rev ish enh(5)	rev ish dim(3p,3q13.3q23,22q13)	63	6	2	1	x	x	Female
c22	rev ish enh(2p25q32,3q11q29,5q22q35,7q21q36,8q,16)	rev ish dim(3p26p12,8p,9,10,13,14,15,17p,18,19)	69	13	2	2	1	x	Female
c23	rev ish enh(8,10,11p15q22,12q11q23)	No imbalance detected	76	13.5	4	3a	x	x	Male
c24	rev ish enh(1q,3q13.3q24,10,17,19)	rev ish dim(3p26p22,3p12,4)	76	7	3	3b	0	0	Male
c25	rev ish enh(5)	rev ish dim(2q36q37,3p)	64	10	1	2	0	x	Female
c26	rev ish enh(5,9p)	rev ish dim(3p,6p25p23,6q21q27,9q)	65	6.5	3	1	x	x	Female
c27	rev ish enh(5q31q35,19p,20q12q13.3,Y)	rev ish dim(3p,3q11.1q13.3,6q22q27,9,14q, 15q12q21)	68	6	2	3b	0	x	Male
c28	rev ish enh(1,2,4q23q35,5,7,8q24.1q24.3,9q,12,13q, 15,16,17,19,20)	rev ish dim(3p26p24,6,9p24p22)	71	7.5	2	3a	0	x	Female
c29	rev ish enh(7,Xq26q28)	rev ish dim(3p21p26)	84	4	2	1	x	x	Male
c30	rev ish enh(5)	rev ish dim(3p26q13.3)	37	11	1	2	x	x	Male
c31	rev ish enh(1q21q23,4q28q35,5q31q35)	rev ish dim(3p26p21,7q31q36)	31	5.5	2	1	x	x	Female
c32	No imbalance detected	No imbalance detected	65	8	2	2	0	x	Male
c33	No imbalance detected	No imbalance detected	69	4.3	2	1	0	x	Female
c34	rev ish enh(2q36q37,7q32q36)	rev ish dim(3p26p21)	63	4	1	1	x	x	Female
c35	rev ish enh(5q23q35)	rev ish dim(3p26p14,Y)	44	6.5	2	1	x	x	Male
c36	rev ish enh(3q,5,7,12,16)	rev ish dim(1p36.3p36.1,4q,9,14q31q32)	55	12	3	3b	0	x	Male
c37	rev ish enh(1q21q41,2q24q33,6p,7,8q21.1q24.3, 9p13q34,10q,16p,17q,19,20q)	rev ish dim(3p26p14,4p16p15.1,4q,5p15.3p15.1, 8p23q13,9p24p21,14q,17p,18,Y)	38	7.5	3	3b	0	x	Male
c38	rev ish enh(5q)	rev ish dim(3p26p22)	72	4.5	2	1	x	x	Female
c39	rev ish enh(3p,6p)	rev ish dim(6q)	87	7	3	3a	0	x	Female
c40	rev ish enh(5,7,11,16,20)	No imbalance detected	66	5	2	1	0	x	Male
c41	rev ish enh(5p13q32,6p21.1q24)	rev ish dim(19)	54	6.3	2	3b	0	x	Male
c42	No imbalance detected	rev ish dim(1q21,16p,19,22)	82	5	3	3a	2	x	Female
c43	rev ish enh(1q21,3q12q21,X,Y)	rev ish dim(1p36.3p31,2q34q37,3p26p21,14q24, 22q13)	81	6	2	1	0	x	Male
c44	rev ish enh(1,5,22q13)	rev ish dim(3p26p13,6q13q27,14q,21q)	78	4.5	2	1	x	x	Female
c45	rev ish enh(1q23q44,2q14.1q33,5p15.3q23)	rev ish dim(1p,3p26q24,3q27q29,8p,11p,14q22q32)	88	8	2	3a	0	x	Male
c46	No imbalance detected	No imbalance detected	90	5.5	3	3a	x	x	Female

Results

In ccRCC, gains of chromosomes 5 (63%); 7 (35%); 1q (33%); 2 (24%); 8q, 12, and 20 (20% each); 3 or 3q (17%); 16 (15%); 19 (13%); and 6 and 17 (11%) represented the most frequent alterations. No amplifications above the threshold tumor-to-normal fluorescence ratio of 1.5 were observed. Most common losses in ccRCC were seen on chromosome 3p or the entire chromosome 3 (63%), 14q (30%), 9 (26%), 1p and 6q or 6 (17%), and 4 and 8p (15% each). Although pRCCs also showed copy number losses, they were less frequent than gains. Most gains were seen on chromosomes 7, 17, 16, and 17. The CGH results for ccRCC and pRCC are summarized in Tables 1–3 and Figs. 1–3.

Correlation of clinical/histopathologic data with genomic imbalances

Contingency table analysis using Fisher's exact test was applied to test the correlation of chromosome imbalances with clinicopathological features. Probability values were calculated using *GraphPad Prism* version 2.01 (GraphPad Software). Statistical significance was set at $P < 0.05$ (Table 4).

Table 3. Chromosome imbalances (ISCN [1995] nomenclature) and clinicopathological data on 15 cases of papillary renal cell carcinoma

Case	Copy number gain	Copy number loss	Age at surgery (yr)	Tumor diameter (cm)	Grade	T	N	M	Gender
p1	rev ish enh(3,4,5,7,9,11,14,16,19,20)	rev ish dim(1,2,6,13,17,21)	53	6.5	3	3a	x	x	Male
p2	rev ish enh(5q,7)	rev ish dim(3p26p13)	52	3.7	1	1	x	x	Male
p3	rev ish enh(7,17q)	No imbalance detected	65	7.5	3	3a	x	x	Male
p4	rev ish enh(12)	rev ish dim(1,14,15,Y)	70	8.5	2	2	x	x	Male
p5	rev ish enh(7,16,21)	No imbalance detected	61	3.2	2	1	x	x	Female
p6	rev ish enh(3q24q29,12q22q24.3,16,17,20)	rev ish dim(14q)	81	4	2	1	x	x	Female
p7	rev ish enh(7p15q36,12q,17,20)	No imbalance detected	62	3.2	2	1	x	x	Male
p8	rev ish enh(1p36.3p32,17)	No imbalance detected	45	5	1	1	x	x	Male
p9	rev ish enh(3,7,10,12,13,16,17,20)	rev ish dim(4q, 5p15.3q23,Y)	51	6.5	3	1	x	x	Male
p10	rev ish enh(1p22p11,3p26p22,3p21.2q13.3,16)	rev ish dim(1p36.3p31.3,6,11q,14q11.2q24.1, 15q11.1q14,Y)	62	6	3	3	2	x	Male
p11	rev ish enh(7,17,20)	No imbalance detected	29	2.5	2	1	x	x	Female
p12	No imbalance detected	No imbalance detected	49	5.5	3	1	x	x	Male
p13	rev ish enh(1p36p32,8,9q34,12q24.1q24.3,15q21q23, 16p,17p,19,20q,22q) rev ish amp (2q22q33,16q,17q,X)	rev ish dim(2q34q37,4p15.3q35,7q,13q22, 15q11.1q21,15q24q26)	69	5	3	1	0	x	Female
p14	rev ish enh(2p16q35,4p15.1q32,5,13q14q21)	rev ish dim(18,19)	49	4.5	2	3a	x	x	Male
p15	No imbalance detected	No imbalance detected	73	12	2	2	0	x	Male

Table 4. Contingency tables of significant correlations in clear-cell renal cell carcinoma (ccRCC)[a]

	Gain of chr 10	No gain of chr 10
g1/g2	0.0	33.0
g3/g4	4.0	9.0

$P = 0.0044$

	Gain of chr 5	No gain of chr 5
g1/g2	24	9
g3/g4	5	8

$P = 0.0441$

	Loss of chr 4	No loss of chr 4
SI/SII	1.0	25.0
SIII/SIV	6.0	14.0

$P = 0.0326$

	Gain of chr 17	No gain of chr 17
g1/g2	1.0	32.0
g3/g4	4.0	9.0

$P = 0.0182$

[a] Probability values *(P)* were calculated using Fisher's exact test. Gain of chromosome 5 correlated positively with low tumor grade (g). Gains of chromosomes 10 and 17 correlated positively with high tumor grade; loss of chromosome 4 correlated positively with disease stage (S).

ccRCCs. Statistical evaluation showed a positive correlation of chromosome 4 loss with tumor stage ($P = 0.0326$). A gain in chromosome 5 ($P = 0.0441$) was inversely correlated with tumor grade, whereas gains in chromosomes 10 and 17 were directly correlated with high tumor grade ($P = 0.0044$ and $P = 0.0182$, respectively).

pRCCs. No significant correlation was found.

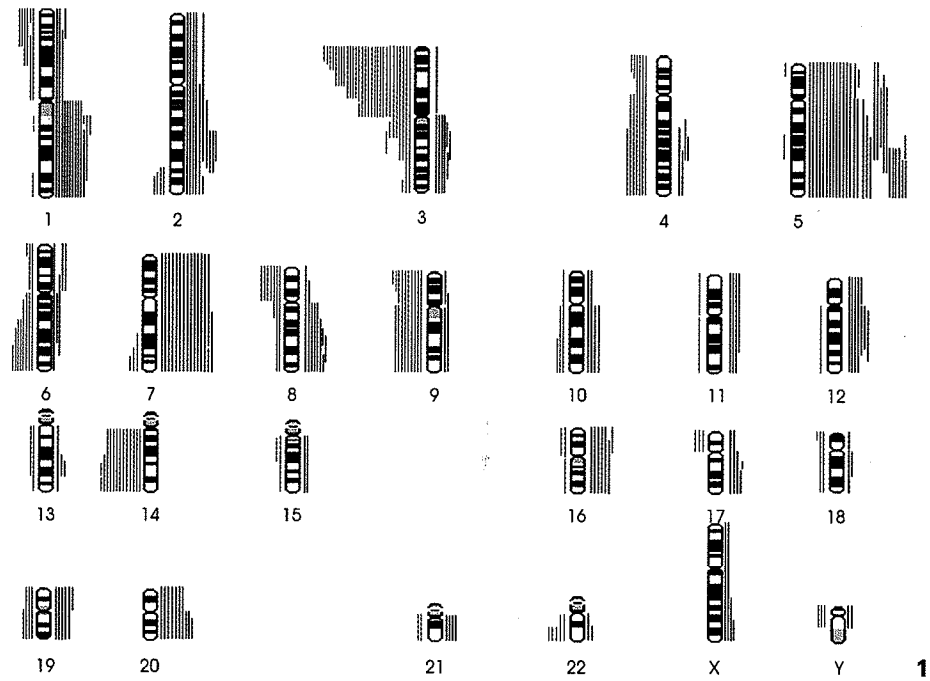
Clinical outcome

Follow-up data were available from 38 patients. Median follow-up was 45 mo, ranging from 1–114 mo. During follow-up, 10 patients with ccRCC and 5 patients with pRCC died. In patients with ccRCC, relapses ($N = 4$) and metastases ($N = 5$) were the cause of death. Four patients with pRCC died of metastatic disease. In pRCC no local relapse was seen. Metastases in ccRCCs occurred in bone ($N = 2$), as well as the lungs and liver (one each). In one patient with ccRCC, the metastasis site was unknown. In pRCCs, metastases were found in bone and lung (one each). One metastasis was found in the para-aortal region, and one metastasis remains unspecified. Tumors in 5/11 patients dying of relapses or metastasis showed loss of chromosome 14q. A contingency table analysis was performed to test the association between chromosome 14 loss or retention with survival. A significant correlation was not seen. A Kaplan-Meier analysis was done to estimate survival probabilities with respect to chromosome changes in each subtype (Kaplan and Meier, 1999), and a log rank test was performed to evaluate significant differences in survival probability. No correlation was seen between chromosome imbalances and survival, patients' age at surgery, or tumor size.

Discussion

Renal cell carcinomas (RCC) are subdivided into five major subgroups based on their histogenetic origin. The clear-cell (ccRCC) and papillary (pRCC) subtypes represent the majority of RCCs. They are derived from the proximal tubulus and account for almost 80% to 85% of all kidney tumors. The clinical outcome of patients with these two subtypes is difficult to predict; however, the occurrence of metastasis is a major predictor of prognosis. Despite the potential for surgical cure, a substantial number of patients will suffer from metastasis and die, even those who underwent radical nephrectomy while no metastases could be detected.

Genetic markers have been suggested to be helpful predictors for the clinical outcome of RCCs (Beroud et al., 1996; Wu et al., 1996). An extensive amount of cytogenetic and molecular studies have provided many subtype-specific genetic markers, as well as prognostic factors. To overcome some of the limitations of conventional cytogenetics and allelotyping approaches,



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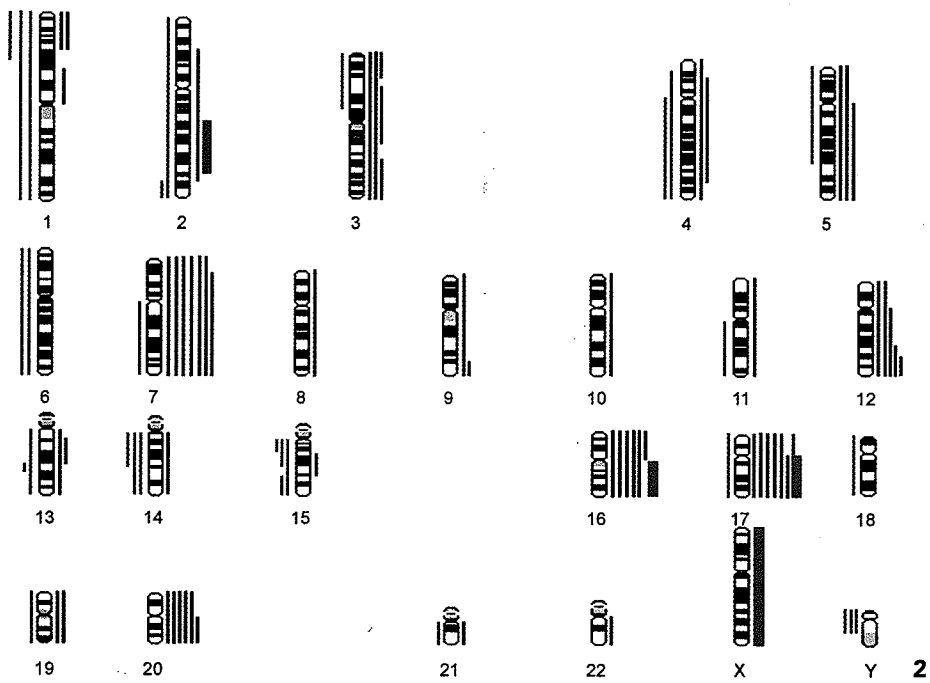

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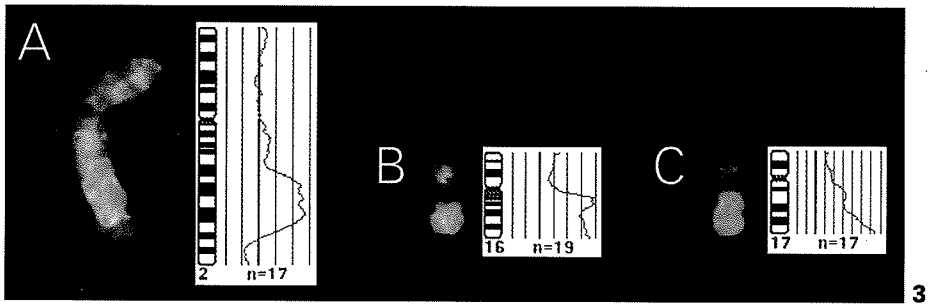

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Fig. 1. Copy number changes in 46 primary ccRCCs. Each bar on either side of the ideograms represents an imbalance. Red bars on the left indicate losses, while green bars on the right show gains.

Fig. 2. Copy number changes in 15 pRCCs. Red bars on the left indicate losses, while green bars on the right show gains. Bars in bold indicate amplified copies.

Fig. 3. Partial karyogram/ideogram with image ratio of CGH experiment p13 (pRCC tumor 13). Red reference fluorescence is overlayed with green test fluorescence (tumor), resulting in greenish subregions, the chromosome arms indicating overrepresentation of tumor DNA. Chromosomes 2 (**A**), 16 (**B**), and 17 (**C**) are shown. The fluorescent intensity ratio profile is given on the right side; the central black line indicates a 1:1 ratio (reference DNA to tumor DNA). The thresholds for under-representation (red lines) and over-representation (green lines) of tumor DNA are also shown. The threshold for chromosome gain was set at 1.25 (the first green line beside the mode line). Ratios exceeding the 1.5 threshold (second green line) were classified as amplifications. Red lines on the left of the black line represent fluorescence ratios of 0.75 (the threshold for copy number loss) and 0.5, respectively.

many workers have turned to CGH in recent years (Speicher et al., 1994; Bentz et al., 1996; Moch et al., 1996; Gronwald et al., 1997; Jiang et al., 1998). However, CGH has its own considerable limitations and is therefore considered to be a complementary, add-on technique to be used in conjunction with more conventional procedures.

We performed a CGH study on 61 RCCs from the proximal tubulus to test whether it was possible to define genetic changes that correlated with the clinicopathological features of these tumors. We were able to establish a significant correlation between genomic imbalances and histopathological and clinical characteristics showing a significant predictive value.

Finding genetic alteration by CGH, we confirmed the majority of genetic changes which have been well established, such as the loss of chromosome 3p at various sites and the gain of chromosome 5q in ccRCCs, and the gain and losses of several chromosomes in pRCCs, as well as ccRCCs (Bugert et al., 1998; Kovacs et al., 1987; Kovacs and Brusa, 1988; Jiang et al., 2000).

The gain of chromosome 5q in ccRCCs was an interesting finding of our study, as it showed a positive correlation with low nuclear grade. This supports the result of a recent study by Junker et al. (2000). Comparing the occurrence of metastasis and primary tumors in ccRCCs, they found 40% non-metastatic tumors with a gain of chromosome 5q versus only 12% in the metastatic group. Although cytogenetic studies showed a gain of chromosome 5q as a common finding in ccRCCs, our study underscores the essence of the former study, that tumors with gain of chromosome 5q might have a reduced metastatic potential, being of lower tumor grade.

Our study presents additional evidence on a significant correlation of genetic findings with histopathological features. Gains of chromosomes 10 and 17 correlated positively with an advanced tumor grade ($P = 0.044$ and $P = 0.0182$, respectively). Both findings have been described in ccRCCs (Elfving et al., 1997; Gronwald et al., 1997; Junker et al., 2000); however, this is the first report to correlate both markers with a high tumor grade.

Loss of chromosome 4 showed a positive correlation with tumor stage ($P = 0.0326$). The finding of chromosome 4 loss in ccRCC might therefore indicate a more advanced tumor. Whether it is an independent prognostic factor needs to be evaluated in studies having a larger sample size.

One pRCC showed an amplification of the chromosome region 2q22→q33. Gene amplification is highly indicative of the presence of an oncogene; it is therefore possible that a protooncogene relevant to pRCC carcinogenesis might be located in the region 2q22→q33.

Despite several prior cytogenetic and allelotyping studies on RCCs, our investigation of these tumors with CGH provides new evidence of significant genetic features that have both clinical and prognostic relevance.

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