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Familial and Sporadic Renal Oncocytomas – A Comparative Molecular-Genetic Analysis

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Key Words

Kidney cancer · Oncocytoma · Genetics · Comparative genomic hybridization · Familial oncocytoma

Abstract

Objectives: Genetic causes of sporadic and familial renal oncocytomas are not known. We analyzed these tumors genetically in order to detect tumor-specific chromosome alterations.

Methods: DNA from 26 sporadic and 31 familial renal oncocytomas were screened by comparative genomic hybridization according to standard protocols including degenerate oligonucleotide-primed PCR.

Results: Chromosome alterations were detected in 19/26 sporadic (73%) and in 4/31 familial renal oncocytomas (13%). Partial or complete losses of chromosome 1 were most frequently found in both sporadic (15/26) and familial tumors (2/4). Less frequently, loss of chromosome 14 (3/26) was detected in sporadic renal oncocytomas as well as losses of 2p, 2q, 4q, 10 and 18 and gains of 1q and 17q in individual sporadic tumors. Inter-tumor variation of chromosome aberrations was prominent in 1 patient, where 1 tumor showed gains of chromosomes 5, 6q, 7, 10p, 12 and 13q, whereas the second tumor exhibited gains of chromosomes 5 and 7 and loss of 10q. In contrast to sporadic renal oncocytomas, most familial tumors (87%) were devoid of chromosome instabilities.

Conclusion: Our results demonstrate that partial or complete loss of chromosome 1 is the most common alteration in renal oncocytomas, sporadic and familial. However, chromosome changes are much rarer in familial than in sporadic renal oncocytomas.

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Introduction

Tumors of the kidney account for 3% of human neoplasms. Recently, a classification system has been introduced subdividing malignant renal parenchymal neoplasms into conventional clear cell, papillary, chromophobe renal-cell carcinoma (RCC), collecting-duct carcinoma and unclassified RCC [1]. Benign parenchymal neoplasms are oncocytic and metanephrogenic adenomas as well as papillary adenomas [2–4]. These subtypes also differ in their biological behavior, and tumor typing is therefore pivotal for patient management [2]. However, histopathological examination does not make it possible to distinguish subtypes considering biological staging and grading of RCC which are essential for individual prognosis. Therefore, genetic investigations were performed to differentiate renal tumors. These analyses revealed specific genetic alterations for subtypes of renal tumors and confirmed the histological classification system. Accordingly, conventional clear-cell RCC is characterized by loss in 3p [5]. Papillary RCC exhibit gains of chromosomes 7, 16 and 17 and loss of the Y chromosome and, less frequently, gains of chromosomes 3, 12 and 20 [6]. Chromophobe RCC are characterized by combined monosomies of chromosomes 1, 2, 6, 10, 13, 17 and 21 [7–9]. Genetic data on collecting-duct carcinomas are still controversial.

Renal adenomas have not been analyzed satisfactorily, partly due to their small size and haphazard sampling. Still, the genetic analysis of renal adenomas may reveal cumulative genetic alterations as conceptualized in the pathogenetic model of a renal adenoma-carcinoma sequence [3].

Renal oncocytomas (RO) are benign epithelial kidney tumors and account for 5% of renal tumors [10, 11]. RO occur both sporadically and hereditarily. Familial renal oncocytomas (FRO) have recently been described in 5 families, where members in 2 or 3 generations were affected by multiple bilateral RO [12]. Sporadic RO are solitary and asymptomatic in most cases and often detected incidentally. Sporadic RO and FRO exhibit similarities, both morphologically and clinically. The genetic basis for the development of RO is not well understood, especially in the familial forms. A limited number of sporadic RO were analyzed by cytogenetic and molecular techniques. These data indicate three tumor subtypes: tumors with loss of chromosomes 1/1p and in some cases combined with losses of chromosomes 14 and Y; RO with translocations involving chromosome 11, and RO with random chromosome alterations or without changes [13–17].

We analyzed sporadic and familial RO by comparative genomic hybridization (CGH) in order to detect chromosome patterns.

Materials and Methods

Patients

Thirty-one tumors from 7 patients in 5 families with FRO were included in this study [12]. The number of affected members in the families ranged from 2 to 4. The median age at diagnosis was 55.8 years. Tumors were detected incidentally in almost all cases. The number of tumors per individual ranged from 1 to more than 10 in both kidneys.

In parallel, 25 tumors obtained from 21 patients with sporadic RO were analyzed. The median age at diagnosis was 67.3 years. In 19 patients, tumors were solitary. Two patients had bilateral multifocal tumors but no familial history of RO.

Pathology

The histology of FRO had been reviewed previously [12]. Slides from sporadic RO were reviewed by two pathologists independently. The diagnosis of RO was based on histopathological criteria established by Amin et al. [10].

DNA Extraction

Tumor tissues were dissected from paraffin sections, and DNA was extracted using a commercial kit (Qiagen) Normal DNA was isolated from blood cells collected from normal individuals using the same kit.

Amplification and Labeling of DNA

In order to obtain sufficient amounts of tumor DNA for CGH analysis, DNA was amplified according to a modified protocol for degenerate oligonucleotide-primed PCR [18, 19]. This protocol employs sequenase during the first 8 cycles of nonspecific PCR, followed by 30 additional cycles under specific conditions using Taq Polymerase (Stoffel fragment). Labeling of tumor DNA and normal DNA was achieved by 20 PCR cycles using biotin-16-dUTP and Digoxigenin-11-dUTP, respectively.

Comparative Genomic Hybridization

One microgram of both tumor DNA and normal DNA was hybridized to 50 μg Cot-1 DNA on normal metaphases at 37 °C for 48 h. Detection of fluorescent signals was carried out with avidin-FITC (tumor DNA) and anti-digoxigenin rhodamine (normal DNA). DAPI-Antifade was used for chromosome counterstaining. Fifteen metaphases were analyzed in each case using an Axioplan microscope (Zeiss Jena, Germany) and a computer system from Metasystems (Altlussheim, Germany). Chromosomal alterations can be detected as shifts of the profile to the red borderline (loss of chromosomal region in the tumor DNA) or to the green borderline (gain of chromosomal region in the tumor DNA).

Results

Twenty-six sporadic RO from 21 patients were analyzed by CGH. Genetic changes were detected in 19/26 tumors (73%). The most common alteration was loss of chromosome arm 1p or the entire chromosome 1 (table 1). Figure 1 demonstrates an example of CGH profile with loss of the short arm of chromosome 1 (1p). This alteration was found

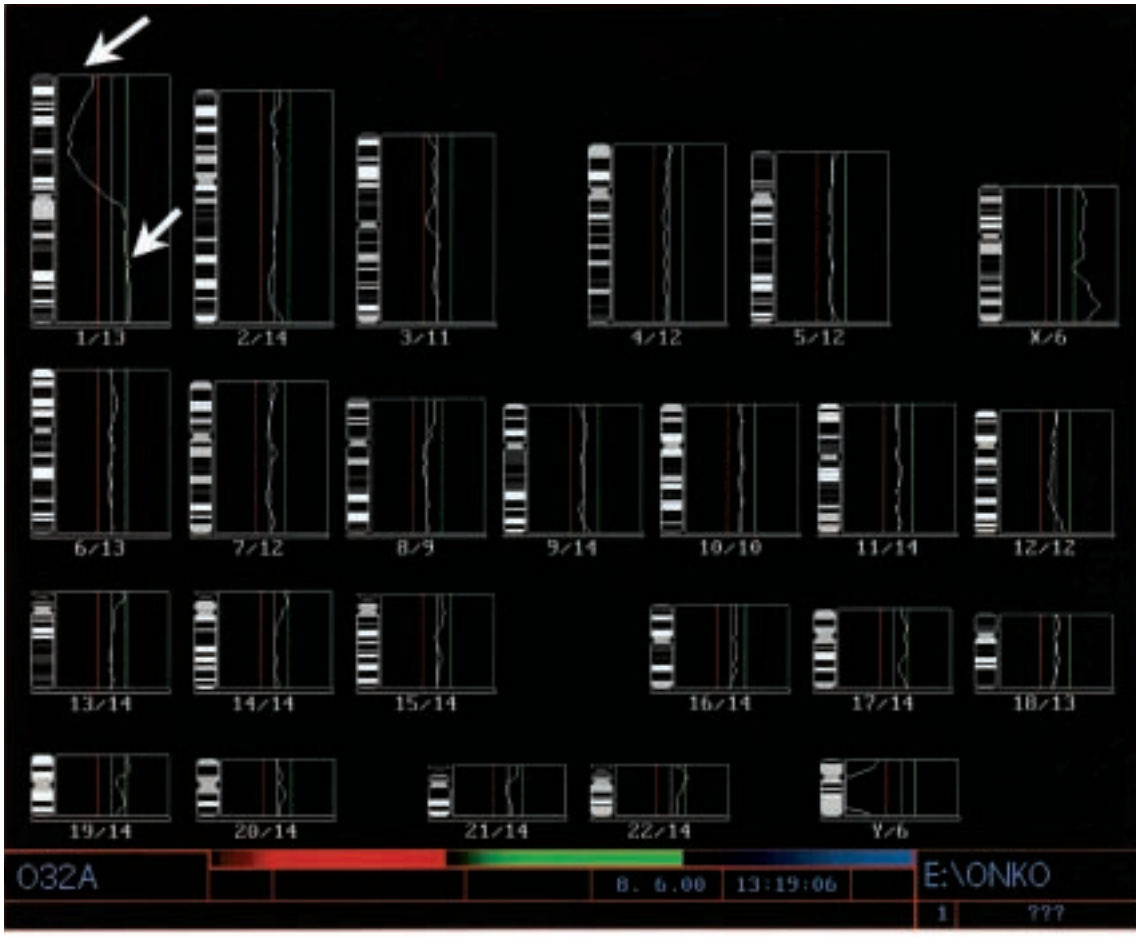

Fig. 1. Fluorescence image obtained by CGH in sporadic RO (20/1): loss of the short arm of chromosome 1 (red), gain of the long arm of chromosome 1 (green), shown by arrows.

Table 1. Genetic alterations detected by CGH in sporadic RO

Patient No.	Age, years	CGH results	Patient No.	Age, years	CGH results
1	64	0	14	75	dim(1, 14)
2	76	enh(17q)	15	91	dim(1)
3	47	dim(1, 18)	16	68	dim(1, 14)
4	62	dim(2q)	17	61	0
5	69	dim(2p, 6q22qter)	18	66	dim(1p, 6q)
6	66	0	19	72	dim(1, 4q)
7	70	enh(17)	20/1	26	dim(1p), enh(1q)
8	63	0	20/2		dim(1p), enh(1q)
9	61	0	20/3		0
10	70	dim(1)	21/1	61	dim(1)
11	61	dim(1)	21/2		dim(1)
12	77	0	21/3		dim(1, 10)
13	63	dim(1, 14)	21/4		dim(1)

0 = No alterations detected; enh = enhancement (gain); dim = diminution (loss).

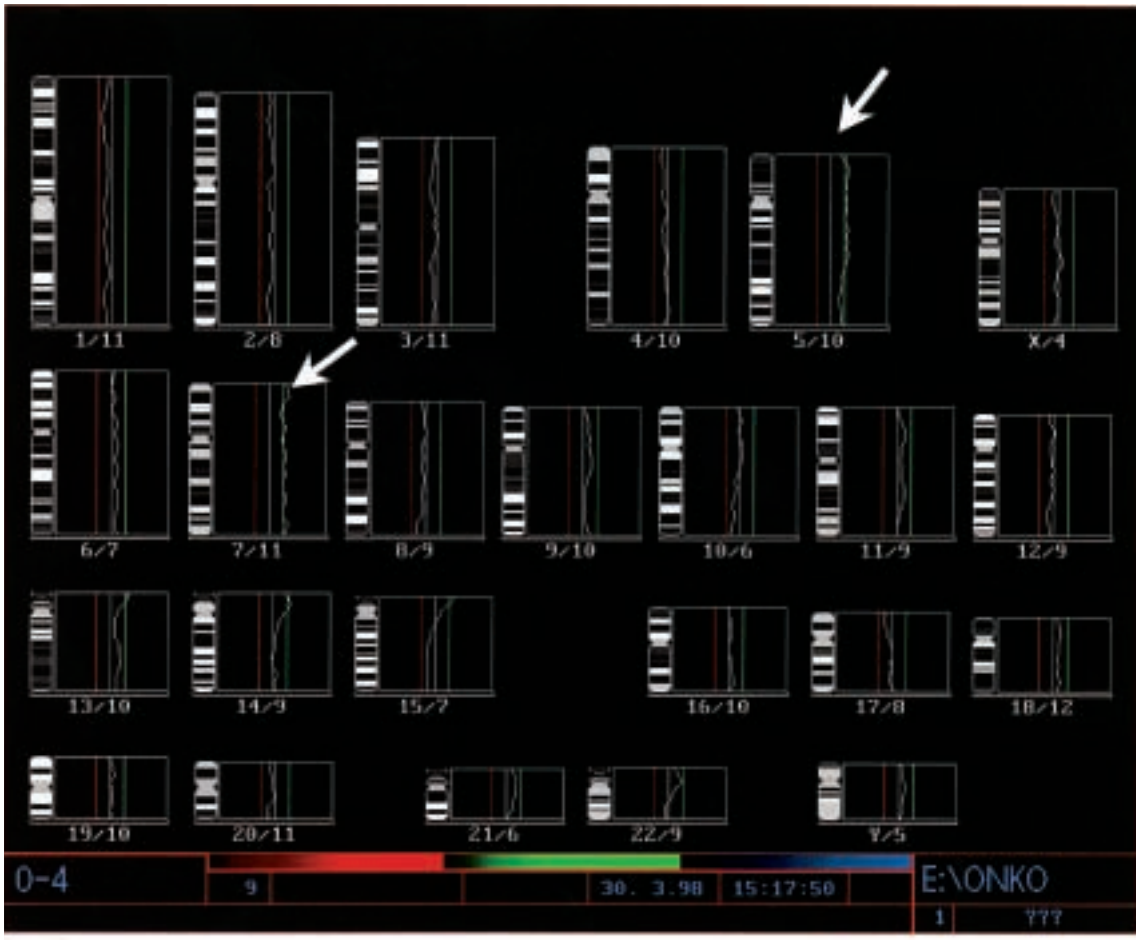

Fig. 2. Image ratios obtained by CGH in a case of FRO (167 II-2): gain of chromosomes 5 and 7 (arrows).

in 15 sporadic tumors (58%). Other aberrations were seen with lower frequencies: loss of chromosomes 14 (3 tumors), 6q (2 tumors), 2p, 2q, 4, 10 and 18 (each in 1 tumor) and gain of chromosome arms 1q and 17q (2 tumors each). Results are presented in detail in table 1. Loss of chromosomes 1, 4, 10, 14 and 18 occurred only in combination with loss of chromosome 1p/1. In 2 patients, bilateral oncocytomas were investigated. Two out of 3 tumors in 1 patient and all tumors in the second patient exhibited identical losses of chromosome 1/1p.

Thirty-one tumors from 7 patients in 5 families were analyzed by CGH. Chromosome alterations were detected in 4/31 (13%) tumors. Tumors from 1 patient showed a complex aberration pattern: 1 RO exhibited gains of chromosomes 5, 6q, 7, 10p, 12 and 13q in different tumor parts (table 2, fig. 2). The contralateral tumor was characterized by gains of chromosomes 5, 7 and loss of 10q. Loss of chro-

Table 2. Genetic alterations detected in FRO from 3 patients

Patient	CGH alterations
167 II-2	
Left	enh(5)
	enh(7)
	enh(6q)
	enh(13q)
	enh(12)
	enh(10p)
Right	enh(5)
	enh(7)
	dim(10q22qter)
169 III-2	dim(1)
170 II-3	dim(1, Y), enh(7)

enh = Enhancement (gain) dim = diminution (loss).

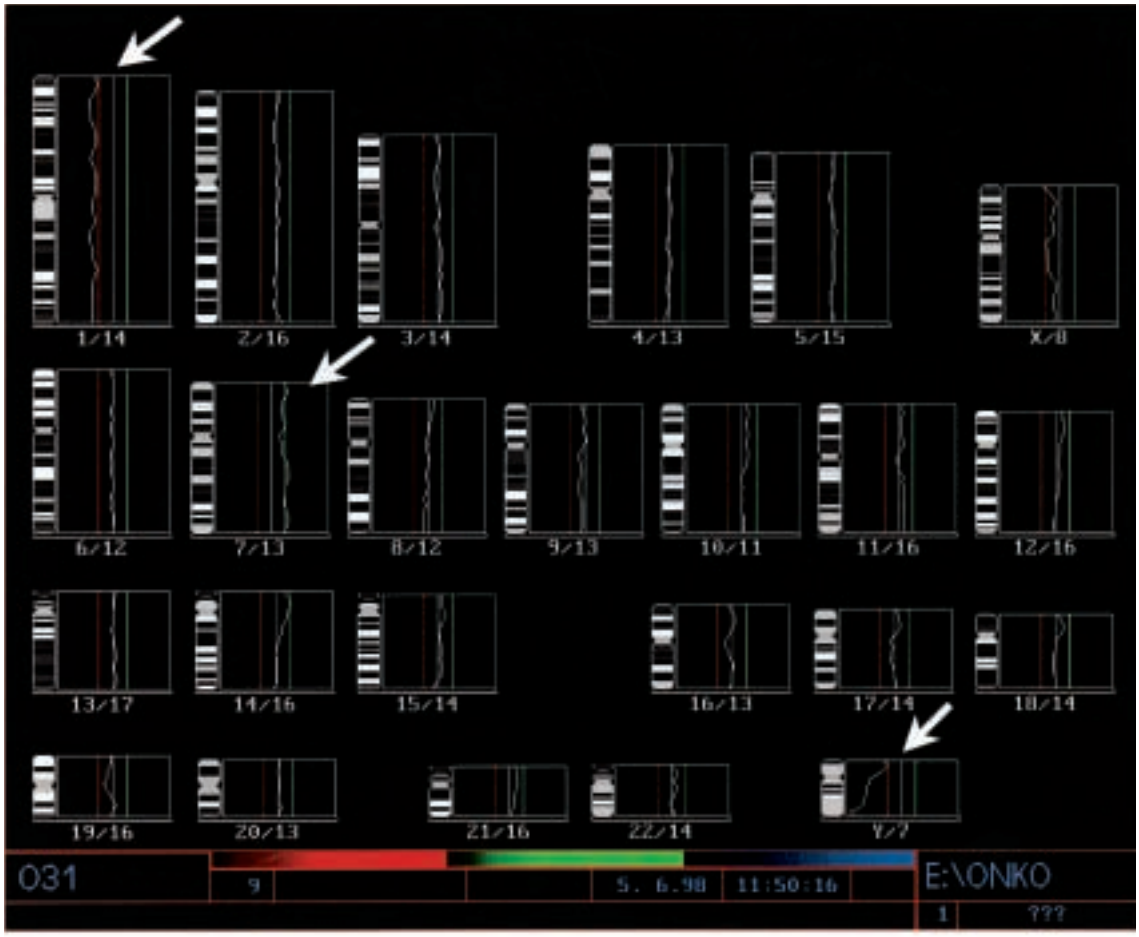

Fig. 3. Image ratios obtained by CGH in a case of FRO (170 II-3): loss of chromosomes 1 and Y and gain of chromosome 7 (arrows).

mosome 1 was found in FRO (2 patients out of 2 families), in 1 patient combined with loss of chromosome Y and gain of chromosome 7 (table 2, fig. 3). Twenty-eight tumors (87%) from other FRO patients were devoid of chromosome instabilities.

Discussion

Renal epithelial tumors represent a heterogeneous group of tumors both histologically and clinically. There are four well-defined subtypes of renal carcinomas: conventional clear-cell RCC, papillary RCC, chromophobe RCC and collecting-duct carcinoma. Benign epithelial lesions of the kidney comprise RO, metanephrogenic adenoma and papillary adenomas. Morphological subtyping of RCC has been helpful for the identification of tumor-specific genetic patterns and the detection of tumor-specific genes. Hence, the key to the identification of the VHL gene in conventional clear-cell RCC, and the MET proto-oncogene in hereditary papillary RCC, was the presence of both inherited and sporadic forms of these renal cancers. Genetic linkage analysis in families with inherited clear-cell RCC and papillary RCC permitted the localization of the disease genes and the identification of mutations responsible for both sporadic and familial forms of these renal neoplasms. The detection of disease-causing genes in chromophobe RCC and RO has been hampered by the lack of hereditary disease variants. This shortcoming has recently been overcome by the identification of families affected by multiple RO [12].

Although RO present characteristic macroscopic and microscopic features, it is often difficult to distinguish between oncocytomas and eosinophilic RCC. They share several biological properties, such as the expression of car-

boanhydrase C, band 3, and cytokeratins [20]. This expression pattern has fostered the hypothesis of a common origin. Genetically, chromophobe RCC show combined monosomies including chromosomes 1, 2, 6, 10, 13, 17 and 21; susceptibility genes have not yet been identified. RO have previously been analyzed by microsatellite analyses of select chromosomal loci and by cytogenetics in single cases, but results are contradictory and may not reflect the status of the entire genome. We therefore scanned DNA from 26 sporadic and 31 familial RO by CGH.

We found that loss of chromosome 1/1p is the most common chromosomal aberration in both sporadic and familial oncocytic tumors. In familial cases, loss of chromosome 1 occurred in tumors from 2 families. Tumors from sporadic cases showed a loss of chromosome 1/1p in 58%. Thus, our results corroborate findings reported by other groups [13, 14, 21, 22] and indicate an involvement of one or more tumor suppressor genes located at chromosome 1p. Unfortunately, based on our data and from other groups obtained by karyotyping and fluorescence in situ hybridization, detection of a specific chromosomal region seems impossible. Thrash-Bingham et al. [22] reported an overlapping region at 1p32pter, Dal Cin et al. [23] a translocation breakpoint at 1p36. In order to pinpoint the chromosomal region and candidate genes involved in tumor development of RO, additional deletion mapping studies seem necessary. The high rate of chromosome 1 losses in sporadic RO point to an early occurrence of this alteration during tumor development, at least in a subgroup of RO. Based on loss of chromosome 1 in RO and chromophobe RCC, Störkel [3] proposed a genetic relationship between both tumors. Additional chromosome alterations in RO would then determine a transition to chromophobe RCC [3].

Loss of chromosome 14 was detected in 3 sporadic cases (12%). This alteration occurred only in combination with loss of 1/1p and may therefore represent a later genetic event. Combined losses of chromosomes 1 and 14 have been reported previously by Presti et al. [21].

Other genetic alterations were rarely seen in sporadic tumors and may represent randomly occurring events not closely linked to the specific genetic milieu in sporadic RO: loss of chromosomes 6q, 2p, 2q, 4, 10 and 18 and gain of chromosome arms 1q and 17q.

In 7 sporadic RO (27%) and 27 FRO (87%), no alterations were detected. This may point to minute chromosome alterations smaller than some megabases in size and/or translocations both not detectable by CGH. Previous cytogenetic and molecular genetic analyses of individual RO, for example, revealed translocations involving chromosome 11 [15–17, 24]. Obviously, cases with these translocations represent a second subgroup of sporadic RO in addition to the group with losses of chromosomes 1, 14 and sex chromosomes [2, 15].

There are several reports about multifocal and bilateral RO suggesting that this is a more common feature in this subgroup of kidney tumors [25–27]. Genetic data from bilateral RO are rare. We analyzed multifocal bilateral tumors from 2 patients without a family history of RO and found identical aberrations of chromosome 1 in different tumors from each patient. The age of 1 patient was 26 years which is strongly suggestive of hereditary disease. However, it seems likely that without a thorough clinico-radiological examination, affected first-degree relatives may not be identified due to the indolent course of RO.

We performed CGH analysis of 21 sporadic and 31 familial RO in order to reveal a chromosomal fingerprint of both variants, which would verify or falsify morphological similarities. Also, a specific reproducible genetic pattern would allow us to position RO in the coordinate system of renal epithelial tumors. Generally, genetic alterations were much more prominent in sporadic (71%) than in familial RO (13%), for yet unexplained reasons. Loss of the entire chromosome 1 or 1p was detected at a high rate in sporadic RO and predominated in the small group of genetically altered tumors in FRO.

In this study, we used CGH, which allows to screen the entire genome for alterations and helps to detect losses and gains of chromosomal regions in tissues including archival tissue sections overcoming the need for cell cultures. CGH can detect deletions larger than 5–10 Mb. Limitations of this technique are the failure to detect very small alterations or balanced aberrations like chromosomal translocations. However, most solid tumors are characterized by unbalanced genetic alterations like losses and gains of chromosomal regions leading to inactivation of tumor suppressor genes or activation of oncogenes. Furthermore, it is necessary that an aberration is present in more than 60% of the analyzed DNA. Therefore, heterogeneity of samples can decrease sensitivity of CGH. CGH should be used as a screening method at the beginning of genetic investigations to define most frequent alterations in specific tumor types. On the basis of these data, molecular techniques like microsatellite analysis, fluorescence in situ hybridization and sequencing can be performed to analyze these genetic alterations in detail.

Our results indicate that RO, sporadic and familial, exhibit a reproducible genetic pattern, namely loss of chromosome 1 or 1p. However, minute chromosome changes, activating mutations of oncogenes or translocations are probably genetic events that characterize most FRO and are

undetectable by CGH. Linkage analysis of families affected by FRO is in progress in order to identify the responsible gene(s). Thus, it seems possible to distinguish RO forms in the future.

Identifying specific genetic patterns of different renal tumor subtypes concerning their different biological behavior, genetic testing will get a high clinical impact enabling the assessment of individual prognoses for patients with tumors of the kidney.

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