Loss of Heterozygosity Studies and Deletion Mapping Identify Two Putative Chromosome 14q Tumor Suppressor Loci in Renal Oncocytomas

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Abstract

Renal oncocytoma is considered to be a benign tumor that shares some phenotypic features with chromophobe renal cell carcinoma (RCC). Recently, we described high frequencies of allelic loss at 1p, 2p, 6p, 10p, 13q, 14q, 17p, and 21q, which correlate significantly with the chromophobe subtype of RCC. To investigate the genetic relationship between these two entities, we examined 12 oncocytomas for loss of heterozygosity (LOH) at these regions. In addition, we included markers for 3p, 5q, 7q, 11p, and 22q. The only chromosomal region showing similarly high frequencies of allelic loss for both subtypes was 14q. Therefore, a genetic relationship between renal oncocytoma and chromophobe RCC seems questionable. Eight of 12 oncocytomas (67%) showed LOH at 14q, a frequency that was significantly higher ($P < 0.001$, $\chi^2$ test) than the frequencies of LOH in all other regions. To define regions potentially harboring novel tumor suppressor genes, we performed multilocus microsatellite analysis with 13 markers spanning 14q. Interstitial deletions at different regions of 14q were detected, with the highest frequencies at D14S258 (14q23-24.3) and D14S292 (14q32.1-32.2). 14q LOH might be associated with advanced-stage RCCs or other tumors, but it does not seem to indicate progression in oncocytomas. Its role in pathogenesis of renal oncocytoma remains to be clarified.

Introduction

Renal oncocytoma comprises about 5% of all renal tumors (1). It is generally considered to be a benign tumor, although there have been some controversial discussions regarding this (2). Genetically, these tumors have revealed specific alterations that are associated with this type of neoplasm. One subgroup is characterized by a 1p loss, which is frequently associated with Y in males and, to a lesser extent, with X in females (3, 4). Another possible subgroup is defined by translocations involving 5q35 and 11q13 (5). Renal oncocytoma originates from the distal part of the renal tubule, sharing this origin with the chromophobe subtype of renal cell carcinoma (6). Phenotypically, it is characterized by the presence of a high number of large and round mitochondria, which account for its granular appearance. These findings are reflected by an up to 5-fold increase in mtDNA (7). The feature of granularity can be troubling in the discrimination of renal oncocytoma from both the eosinophilic variant of chromophobe RCC and the dedifferentiated granular cell RCC. Recently, the presence of chromophobe cells in an oncocyto has been described, suggesting a close relationship between these two types of renal tumors (8). Apart from these phenotypic similarities, structural alterations in mitochondrial DNA were shown for both chromophobe RCC (9) and oncocyto (10). Here, we examined whether the genetic similarities between renal oncocyto and chromophobe RCC are based on common molecular genetic alterations. In a previous study (11), we found that allelic loss at specific chromosomal regions (1p, 2p, 6p, 10p, 13q, and 21q) correlated significantly and specifically with the chromophobe subtype of RCC. A high frequency of LOH at 14q and 17p was also detected but did not discriminate between chromophobe and clear cell, or chromophobe, RCCs. In addition to these regions, we performed minisatellite analysis for several other loci, adding two microsatellites from the loci D3S1350 (3p) and D5S404 (5q), because loss at these regions has been shown to be relevant for chromophobe RCC (9). Having detected high frequencies of allelic loss at D14S13, we performed a refined deletion mapping of chromosome 14q, using 13 highly polymorphic dinucleotide repeat markers that were evenly spaced along 14q. A fluorescence-based approach, using fluorescently tagged oligonucleotide primers for PCR amplification, was chosen for deletion mapping. Analysis was performed on an ABI 373 DNA sequencer (Applied Biosystems, Foster City, CA). This allowed for simultaneous analysis of 13 loci in a single lane, avoiding the disadvantages inherent to conventional radioactive analysis (12, 13).

Materials and Methods

Tissue Samples. Paired tumor and normal tissues from 12 patients were obtained after nephrectomy from the Department of Urology, Johannes Gutenberg University Clinics (Mainz, Germany), and were immediately snap-frozen. The material was stored at −80°C until isolation of genomic DNA. Cellular typing was performed according to the criteria established for the histogenetic classification (1). Informed consent was received from all patients prior to nephrectomy.

Probes. DNA probes were obtained from the American Type Culture Collection (Rockville, MD). For probe data, see Table 1.

DNA Isolation and Minisatellite Analysis. High molecular weight DNA was isolated according to a standard phenol-chloroform extraction procedure, followed by ethanol precipitation. Southern blots were performed as described previously (14).

Radioactive MSA. Allele status at D3S1350 and D5S404 was determined using radioactive MSA. PCR products were separated on a 6% denaturing urea-polyacrylamide gel and Southern blotted onto a Hybond N+ nylon membrane (Amersham, Buckinghamshire, England). A mixture containing 100 ng of one primer per pair was radioactively end-labeled with $[^{32}P]_{-}ATP$ (Amersham) and used to probe the nylon filter in Church solution (7% SDS, 0.5 mM Na2HPO4, and 1 mM EDTA). Hybridization was carried out overnight at 42°C. Washes were 5 min in 5× saline-sodium phosphate-EDETA and 0.5% SDS at 40°C and 2 min in 2× saline-sodium phosphate-EDETA and 0.5% SDS at room temperature. The filter was exposed to BIOMAX MR Film (Eastman Kodak, Rochester, NY) for 24 h at −80°C and then developed. Allelic loss was scored visually by two independent observers (compare Fig. 1).

Received 8/18/97, accepted 10/2/97.

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1 This work was supported by the Deutsche Forschungsgemeinschaft (Grant De 356/1-2).

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3 The abbreviations used are: RCC, renal cell carcinoma; LOH, loss of heterozygosity; MSA, microsatellite analysis; TSG, tumor suppressor gene.
14q Microsatellite Loci and Primers. Thirteen microsatellite (dinucleotide repeat) loci along chromosome arm 14, with an average spacing of 9.2 cM, were examined. Twelve primer pairs that had been redesigned for optimal performance were selected from the Human Linkage Mapping Set Panel 20 (Applied Biosystems), and for qter coverage, the terminal marker SCW1 (15) at D14S526 was selected. One primer per pair was fluorescently labeled with one of three Applied Biosystems dyes (6-FAM, HEX, and TET). All primer sequences can be obtained from Research Genetics (Huntsville, AL). Data on the map location of the markers used are accessible through the Center of Genome Research at the Whitehead Institute for Biomedical Research (Cambridge, MA; http://www.genome.wi.mit.edu).

Fluorescent PCR and PAGE. PCRs were performed in a total volume of 30 μl, containing 200 ng of genomic DNA in a solution of 50 mm Tris-HCl (pH 8.5), 15 mm (NH₄)₂SO₄, 3.5 mm MgCl₂, and 1 unit of PfuTurbo DNA polymerase (PanSystems, Nuremberg, Germany). Multiplexing of PCR products and sample preparation were carried out according to the instructions supplied by the manufacturer (Applied Biosystems). The samples were electrophoresed at 30 W in a 6% denaturing urea-polyacrylamide gel for 1 h using an ABI 373A automated DNA sequencer (Applied Biosystems). For case 3, there was no material left to perform the MSA.

Assessment of Allelic Loss. The imbalance factor, defined as the ratio of allele intensity in a tumor sample relative to the ratio of allele intensity in the corresponding normal DNA, was calculated using the peak height data generated by the Genescan 620 analysis software (Applied Biosystems). In accordance with a previous study (16), the cutoff value of 1.3 was chosen for scoring of allelic loss.

Comparison of Allelic Loss. A modification of comparative multiplex PCR analysis (17) was used. Reference markers for a locus showing homozygosity and the locus in question were coamplified. A reduction of signal intensity of about 50% in the tumor allele relative to the corresponding normal allele indicated hemizygous loss.

Results and Discussion

To investigate the genetic relationship between renal oncocytomas and renal chromophobe tumors, we performed LOH studies on oncocytomas using polymorphic minisatellites/microsatellites for 13 loci of different chromosomes. To determine the presence of novel TSGs on 14q, multifluorescence MSA of 13 loci that were evenly spaced along 14q was done.

We analyzed normal and tumor DNA from 12 renal oncocytomas for LOH, applying radioactive minisatellite analysis/MSA. As for chromophobe tumors, a specific pattern of loss of chromosomes 1, 2, 6, 10, 13, 17, and 21 has been established (9, 11, 18), and we used probes from these chromosomes and included markers from the chromosomal regions 3p, 5q, 7q, 11p, 14q, and 22q, also known to be important for renal tumorigenesis. Typical autoradiograms are shown in Fig. 1. The clinical data, probes applied, and results of LOH analysis are listed in Table 1. For comparison, the results of a recent LOH study by Polascik et al. (19), as well as the combined results, are given. Our LOH study revealed a high frequency of LOH at 14q. Eight of 12 (67%) tumors that were informative at D14S13 showed loss of DNA fragments from 14q. Including the results from the subsequent MSA analysis, the percentage of tumors with loss at 14q was even higher: 10 of 12 (83%) tumors showed loss at one or more loci on chromosome 14. This frequency was significantly higher (P < 0.001, χ² test) than that of loss at any other chromosomal region tested.

Previous cytogenetic analyses failed to detect 14q deletions in oncocytomas (5). As in our study, no tumor showed loss of the entire chromosome 14 instead of interstitial deletions, and this reflects the limitations of conventional cytogenetics in detecting submicroscopic lesions and underscores the importance of complementary molecular studies, especially in benign tumors such as oncocytomas.
A recent allelotype study of 13 oncocytomas (19) described an accordingly high frequency of allelic loss (total of 46%) for two other microsatellite markers, namely D14S288 (14q13—21) and D14S267 (14q32). Polascik et al. (19) also found a high frequency of LOH at 1p (57%). Only two of eight informative tumors in our study showed LOH at chromosome 1. This difference might be due to the small sample numbers of both studies, especially because there has been evidence that loss of chromosome 1 is more often associated with a male karyotype (20), and in our study, three of eight male samples were uninformative for the 1p locus.

Loss at 14q has been associated with loss at other chromosomal regions in six oncocytomas, whereas only two tumors have shown loss of 14q as the sole change. This may indicate an association between genome instability and 14q LOH, as has been hypothesized by Thrash-Bingham et al. (21). Here, only 14q showed a high LOH frequency that compares well with the frequency seen in the chromophobe subtype (7 of 11 = 64% in our previous study; Ref. 11), applying the same probe. Therefore, a possible genetic relationship between renal oncocytoma and chromophobe RCC remains questionable. Because, on the other hand, 14q LOH may be involved in generating an unstable genome (21) and because, in chromophobe RCC, the low chromosome number that is commonly observed (9) might reflect a genomic instability, oncocytoma cells cannot be excluded as precursors for chromophobe tumor cells.

Frequencies of allelic loss at the other loci did not differ substantially from other LOH studies on oncocytomas published thus far (7, 19, 22). Minor differences might be ascribed to the different locations of the probes used and the small number of cases studied thus far.

To narrow down the minimal deleted regions, we selected 13 highly polymorphic microsatellite markers that were spaced evenly between 14q12 and 14qter. Fig. 2 shows typical electrophoreograms of three tumors. The order of the markers and the results are summarized in Fig. 3. Multifluorescence MSA identified interstitial deletions at various loci. However, at two regions, a higher frequency of loss was seen: D14S258 (three of nine; 33%) located at 14q23—24.3, and D14S292 (four of nine; 44%), at 14q32.1—32.2. The loci retaining heterozygosity were defining the critical intervals on either side; the centromeric region was defined by D14S63 and D14S74, whereas the telomeric one was defined by D14S78 and D14S826. Interestingly, the two tumors (cases 2 and 4) that have been uninformative in the fine-mapping analysis had shown LOH at D14S13, a marker located within the distal critical interval, underscoring the importance of this region. The fact that this minisatellite probe (D14S13) detected a higher frequency of allelic loss (67%) than any other of the microsatellite markers (D14S288, 33%; and D14S292, 44%) may indicate its location directly within the critical distal region at 14q32. Thus far, no TSGs have been localized to these regions.

There has been no evidence for metastases in any of the patients of this study. The clinical outcome was available for 11 patients, with a mean follow-up period of 43 months. No patient died from oncocytoma-related causes. Therefore, LOH at 14q was not suggestive of malignant transformation. (2) Interstitial loss occurred in tumors of small size, as well as in larger, higher-grade tumors, indicating no association with tumor progression. Thus, in 14q has been proposed a progression marker in nonpapillary RCCs because, in these tumors, loss of 14q correlated with high stage and grade, as well as with poor survival (23). Recently, a number of LOH studies focusing on 14q in various RCC subtypes or even other types of tumors have been published. It has been shown that a high frequency of 14q LOH was associated with advanced disease in colorectal carcinomas (24), meningiomas (25), bladder cancer (26), and ovarian carcinomas (27). For bladder and ovarian carcinoma, two critical LOH intervals have been defined. For these two tumor types, the most distal regions of common LOH have been defined on their telomeric side by D14S267. This marker is located only about 4 Mb telomeric to D14S78, which is the proximal boundary of the LOH region of our study. It remains speculative whether this might refer to the same TSG, or whether each type of carcinoma has a specific tumor suppressor. Finally, the identification of the TSGs involved in pathogenesis of renal oncocytoma will clarify whether these genes are also relevant for other RCC subtypes or even other types of tumors.

**Fig. 3.** Deletion map of 14q. Left, markers used and map positions. The critical regions with the highest frequencies of loss in D14S63—D14S74 at 14q23—24.3 and in D14S78—D14S826 at 14q32 are marked. Top, tumor number. □, allelic loss; ■, retention of heterozygosity; □, hemizygous loss; blank, not informative.
Thus far, LOH analysis for multiple markers has only been performed for chromosome 1 in four cases of renal oncocytoma (20), indicating that 1p harbors a TSG. Here, we provide evidence for the presence of two additional TSGs at distinct loci on 14q that are involved in tumorigenesis of renal oncocytomas. The data of our study will be useful for further refining the critical regions and for isolating novel TSGs. This eventually will clarify the role of these genes for development of oncocytomas and chromophobe renal tumors, including their significance for genomic stability in tumors.

References


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