Analysis of Chromosome 1p Abnormalities in Renal Oncocytomas by Loss of Heterozygosity Studies

Correlation With Conventional Cytogenetics and Fluorescence In Situ Hybridization

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Abstract

We previously showed by cytogenetics and fluorescence in situ hybridization (FISH) that the most common chromosomal abnormality in renal oncocytomas is loss of chromosome 1 or 1p. In the present study, we evaluated chromosome 1 by loss of heterozygosity (LOH) studies. DNA was extracted from paraffin sections. Three microsatellite markers were used: D1S508, D1S199, and D1S2734. The regions targeted by FISH probes and LOH markers were close to each other but not overlapping.

Among 16 tumors evaluated by all 3 techniques, in 2 cases, LOH could not be interpreted. LOH was detected in at least 1 locus in 12 (86%) of 14 renal oncocytomas studied, with other loci being noninformative or not interpretable (1 case). In 2 cases, the LOH results were inconclusive.

These results provide further evidence to support widespread abnormalities in chromosome 1p in renal oncocytoma. Determining whether such abnormalities are unique to renal oncocytomas or are also present in other tumors requires further studies.

Renal oncocytoma (RO) is a benign renal epithelial neoplasm that comprises 5% of renal tubular epithelial neoplasms. It is composed of large cells with mitochondria-rich eosinophilic cytoplasm.1 Although patients in most series show a wide age distribution at presentation, the peak incidence is in the seventh decade of life.1 Males are affected almost twice as often as females. ROs arise from intercalated cells.1 The relationship between ROs and other renal tumors derived from the distal nephron remains an open question.2,3

Currently, according to the Mitelman Database of Chromosome Aberrations in Cancer karyotypic data have been reported for 81 ROs.4 Cytogenetic studies showed considerable heterogeneity among oncocytomas. Although a subset of tumors showed a normal karyotype, some tumors showed 1 or more of the following: loss of chromosome 1, loss of the short arm of chromosome 1 (1p), loss of the Y chromosome (in male patients), and occasionally, monosomy of chromosome 14; a few tumors were reported to have structural rearrangements involving chromosome 11 band q13.1,2,5-7 We previously showed that the most common chromosomal abnormality in renal oncocytomas is loss of chromosome 1 or 1p, which was detected in 32% of tumors by cytogenetics and in 59% by fluorescence in situ hybridization (FISH).5,7 Although the rate of detection of chromosome 1 abnormalities was higher with FISH studies, we did not observe any isolated deletion of 1p36.3 in many tumors in which both chromosomes 1 were normal by conventional cytogenetics. In the present study, we sought to look for chromosome 1 abnormalities by loss of heterozygosity (LOH) studies using 3 markers that are located close to (but not overlapping) the prior subtelomeric 1p FISH probe. We also sought to compare the results of 3 techniques (conventional cytogenetics, FISH, and LOH) in the evaluation of chromosome 1 in ROs.
Materials and Methods

This study was approved by the Loyola University Medical Center institutional review board. Sixteen tumors were analyzed using LOH. The results of conventional cytogenetics and FISH were previously reported.5,7 There were 10 men and 6 women, with ages ranging from 42 to 84 years (mean, 65 years). The histopathologic diagnosis of renal RO was based on the 2004 World Health Organization classification of renal epithelial tumors1 and specifically included histochemistry (Hale colloidal iron), immunohistochemistry (cytokeratin 7, CD10, CD117, and vimentin), and electron microscopy (abundant mitochondria) as previously described.8 None of the patients had a family history of RO. Sixteen tumors were evaluated by all 3 techniques. In 2 cases, LOH studies could not be interpreted.

Paraffin DNA Extraction

Unstained paraffin-embedded tissue slides were heated at 60°C for 30 minutes and then treated with xylene twice for 10 minutes each. After air drying for roughly 1.5 hours, the paraffin became visibly white. By superimposing the unstained slide on top of a marked stained slide, areas of tumor and normal tissue were scraped off the slide and placed into microfuge tubes. Cold extraction buffer (10 mmol/L of tris(hydroxymethyl) aminomethane hydrochloride [pH 8.0], 1 mmol/L of EDTA, and 1% polysorbate 20) was added in volumes of 50 to 150 µL, depending on the amount of material removed from the slide, and supplemented with 0.1% Proteinase K. Samples were incubated at 52°C for 14 to 16 hours followed by heat shocking at 96°C for 8 minutes to inactivate the Proteinase K. The resulting solution was used as a template for polymerase chain reaction (PCR) with no additional purification steps. Concentration was determined by using a Hoefer DyNA Quant 200 fluorometer according to the manufacturer’s instructions (Amersham Biosciences, Piscataway, NJ).

Amplification

The 3-marker microsatellite panel consisted of the following markers: D1S508, D1S199, and D1S2734. All primer pairs (Applied Biosystems, Foster City, CA) consisted of a forward primer labeled with a fluorescent tag and a “tailed” reverse primer designed to promote consistent polyadenylation of PCR products. Reactions were not multiplexed. A hot start was used to prevent nonspecific amplification, and thermocycler conditions (95°C for 1 minute, 57°C for 1 minute, and 72°C for 1 minute) were repeated for 40 cycles, followed by a final extension of 30 minutes to promote the formation of full-length product and minimize stutter peaks.

Fragment Analysis

PCR products were diluted 1:10 and combined with Hi-Di Formamide (Applied Biosystems) and Genescan 350 (Applied Biosystems) and then denatured at 95°C for 2 minutes and ramped to a 4°C holding temperature before loading on the 3100 Avant Genetic Analyzer (Applied Biosystems).

Peak Interpretation

Peak interpretation is illustrated in Figure 1. For samples that yielded a heterozygous result, 2 factors were considered: the allele molecular weight and the peak height.
(rather than peak area). HMW/LMW (height of the high molecular weight/height of the low molecular weight) represents a relationship between the 2 peaks that can be used to determine the allelic imbalance suggestive of LOH. For this study, normal ranges were established for each marker (results not shown) by determining the HMW/LMW for a subset of 20 normal patients, ie, those without a diagnosis of RO and not expected to have any allelic imbalance. These ranges acted as guidelines to help detect loss, but true LOH determination was influenced more by comparing the normal area of the slide with the abnormal area of the slide. Here, a difference of 15% or greater between normal and abnormal HMW/LMW values suggested a loss. Because homozygotes have alleles of the same size, their peaks overlap and the HMW/LMW calculation does not apply; therefore, they are considered noninformative results.9

Interpretation of the LOH data was performed without prior knowledge of the cytogenetic and/or FISH results. For FISH studies, we used a subtelomeric 1p36.3 probe (29 megabases from the end of the chromosome, containing TP37 and EGFL3) in tandem with 1q25 (Abbott Molecular, Des Plaines, IL). The regions targeted by FISH probes and LOH markers were close to each other but not overlapping 

Figure 2. 

B, A case that shows no LOH has peaks of differing sizes and similar ratios of peak height. NL, HMW/LMW = 49.39%. ABNL, HMW/LMW = 53.39%. C, A case that shows LOH has peaks of differing sizes but dissimilar ratios of peak height. In this example, the height of the HMW peak has dropped, which causes a roughly 10-fold decrease in the ABNL ratio compared with the NL ratio. NL, HMW/LMW = 44.96%. ABNL, HMW/LMW = 4.23%.
Results

Of 16 tumors studied, 14 were successfully analyzed. Of the 14 tumors, 12 (86%) showed LOH in at least 1 locus. Specifically, 7 tumors (cases 1-7) showed LOH in all 3 loci. The results varied in tumors 8 through 14. Cases 8, 9, and 12 through 14 showed LOH in 1 or 2 loci, and the remaining locus (loci) was noninformative or, in 1 tumor, could not be interpreted owing to technical reasons (D1S508 locus). Tumors 10 and 11 showed no detectable LOH in 1 (case 10, D1S508 locus) or 2 loci (case 11, D1S508 and D1S2734 loci), and the remaining markers were noninformative.

Among the 3 loci, D1S2734 showed LOH in 12 tumors, was noninformative in 1 tumor, and no LOH was detectable in 1 tumor. The D1S199 locus showed LOH in 8 tumors, and the remaining tumors were noninformative. The D1S508 showed LOH in 10 tumors, and in 2 tumors, no LOH was detected; the marker was noninformative in 1 case and could not be interpreted in 1 case [Table 1].

There was excellent correlation of cytogenetics, FISH, and LOH data in 7 cases showing loss of 1p or 1. In 3 tumors disomic for chromosome 1 by cytogenetics and monosomic by FISH, LOH was detected in all 3 tumors (by 2 markers, with 1 marker in each tumor being noninformative [D1S508 once and D1S199 twice]). Thus, the LOH study supported the FISH data, suggesting that stromal overgrowth possibly may have influenced cytogenetic evaluation. Interestingly, in 4 tumors with disomy of chromosome 1 by karyotyping and FISH, LOH was detected in 2 tumors in 1 and 2 loci, with the other loci being noninformative (D1S199 twice) or noninterpretable for technical reasons (D1S508 once). In 2 remaining tumors (cases 10 and 11), no LOH was detectable in 1 or 2 loci (D1S508 twice and D1S2734 once), and the other loci were noninformative.

Discussion

Previously, we demonstrated by cytogenetics and/or FISH the existence of high rates of chromosome 1 abnormalities in RO (32% and 59%, respectively). The present study showed LOH within a subtelomeric 1p region in at least 1 locus in 12 (86%) of 14 tumors. Only 2 tumors showed inconclusive results with lack of detectable LOH in 1 or 2 loci in each tumor with the remaining loci being noninformative.

There was excellent correlation of cytogenetics, FISH, and LOH results in 7 (50%) of 14 cases. In 5 additional cases (36%) in which LOH was detected, there was discordance between LOH and cytogenetics and/or FISH, and in 2 cases (14%), the LOH data were inconclusive. Thus, as expected, in cases with −1p and monosomy of 1p36.3 alone and in cases with −1 and a loss of 1p36.3/1q25, there was LOH in all 3 loci. However, in 3 of 14 cases with discordance between karyotyping and FISH results (disomic for chromosome 1 by cytogenetics and monosomy of 1p36.3/1q25 by FISH), there was LOH in 2 loci in each tumor, with the third locus being noninformative. These results suggest that cytogenetic results may have been based on evaluation of overgrown stromal cells in culture rather than tumor cells. Unexpectedly, however, among 4 cases with disomy of chromosome 1 by cytogenetics and disomy of 1p36.3/1q25 probes by FISH,
LOH was detected in 2 cases. These results suggest that “submicroscopic” abnormalities in the LOH regions studied are present in RO with an apparently normal karyotype and that these abnormalities are not detectable by the FISH probes used in previous studies. In 2 remaining cases, the results were inconclusive with no detectable LOH in 2 loci (D1S508 and D1S2734), with the remaining loci being noninformative.

It has been proposed that loss of 1p may be associated with the loss of an as yet unidentified tumor suppressor gene that is important for the genesis of ROs.\(^2,10,11\) Allelic loss within the studied region of chromosome 1p further supports this notion and brings nearer the issue of a delineation of the minimum deletion interval for the proposed tumor suppressor gene. The question arises, “What is the relationship between RO, a benign tumor, and other distal nephron cancers, in particular with chromophobe renal cell carcinoma?”\(^2,3,12-15\)

Morphologic overlap between RO and chromophobe renal cell carcinoma has long been recognized.\(^2,12\) Determining whether LOH within 1p detected in this study is unique to RO or is also present in other tumors, particularly in chromophobe renal cell carcinoma, requires further studies.

The present study provides further evidence to support widespread abnormalities in chromosome 1p in ROs, including those with normal chromosome 1 by conventional cytogenetics. Determining whether such abnormalities are unique to renal oncocytomas or are also present in other tumors, particularly in chromophobe renal cell carcinoma, requires further studies.

**References**


**Table 1**

Results of LOH Studies in 14 Renal Oncocytomas Compared With Results of FISH and Conventional Cytogenetics\(^*\)

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FISH, fluorescence in situ hybridization; LOH, loss of heterozygosity; NI, noninformative; +, presence of LOH; –, no evidence of LOH; ?, not interpretable for technical reasons.

* There was concordance between various methods except where the solid line shows discordance and the dotted line inconclusive results.

† Number of signals present.

‡ Number of chromosome number 1 present.

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