Localisation of Tumor Suppressor Loci on Chromosome 9 in Primary Human Renal Cell Carcinomas

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Abstract

To investigate the potential loss of tumor suppressor gene loci on chromosome 9 in human renal cell tumorigenesis we analyzed 42 paired normal and tumor DNAs with 18 polymorphic microsatellite markers spanning this chromosome. Fourteen of 42 (33%) tumors showed partial or complete deletion of chromosome 9. Detection mapping provided evidence for the presence of a suppressor locus on both the short and long arm of chromosome 9. Homozygous deletion at 9p21–22 in one renal tumor and a selective deletion of distal 9q in another tumor localized the critical regions. The CDKN2/p16 gene was further investigated as a candidate suppressor locus on 9p21–22 by multiplex PCR, Southern analysis, and exon sequencing. We found no additional cases of homozygous deletion, rearrangement, or point mutations of CDKN2/p16. This is the first report of 9p loss of heterozygosity, homozygous deletion of 9p21–22, and selective deletion of 9q in primary renal cell carcinomas. Understanding the molecular genetic basis of renal cell progression will require the isolation and characterization of additional tumor suppressor genes on chromosome 9.

Introduction

Renal cell carcinoma is seen most commonly as a sporadic adult tumor with an incidence of almost 26,000 new cases and 10,000 deaths reported annually in the United States.1 Loss of chromosome 3p occurs frequently in renal cell tumors and represents the most common genetic event described in this cancer (2–4). Deletion-mapping studies of chromosome 3p have revealed several regions of loss on this arm (5, 6). One known target of deletion is the recently cloned gene predisposing to von Hippel-Lindau syndrome (7). Deletions and mutations of this gene, which maps to 3p25–26, have been found to be common in sporadic renal cell carcinomas (8). The chromosomal region 3p12–14 (which contains the familial RCC8 breakpoint) and the 3p21 region are also lost in many renal tumors (5, 6), implying the existence of other tumor suppressor genes on 3p. Because adult sporadic cancers are known to arise from multiple genetic events (9) it is likely that other suppressor loci important in renal cell carcinogenesis remain to be discovered. Allelotype and deletion mapping studies have identified loss of chromosomes 5q, 6q, 10q, 13q, and 17p in approximately 15–30% of sporadic renal cell tumors (4, 10, 11).

Tumor suppressor genes on both arms of chromosome 9 appear to be frequently involved in many types of cancer. Chromosome 9q is frequently deleted in bladder tumors (12, 13), lung tumors (14), and basal cell carcinomas (15, 16). Loss of the p arm of chromosome 9, in particular 9p21–22, is also common in many human tumors. Prior studies have shown the region of 9p21–22 to be deleted in leukemias (17), melanomas (18), gliomas (19), as well as lung (20), bladder (21), and head and neck cancers (22). Furthermore, a candidate suppressor gene, CDKN2/p16, was recently isolated from 9p21 (23, 24) and found to be homozygously deleted in many types of tumor cell lines including some derived from renal cell carcinomas (23). Chromosome 9p has not been studied previously in primary renal cell carcinoma. To examine whether suppressor loci on chromosome 9 are involved in renal cell tumorigenesis, 42 sporadic renal cell carcinomas were screened for LOH at 18 highly informative microsatellite loci spanning the chromosome. We also investigated potential inactivation of CDKN2/p16 by homozygous deletion, rearrangement, or point mutation.

Materials and Methods

Renal Tumor and Constitutional DNAs. Forty-two primary tumor specimens were obtained after nephrectomy and frozen immediately. Thirty-eight tumors were of clear cell type and four were of a mixed clear and granular type. Two of the 42 tumors had a papillary pattern and another 4 showed a mixed papillary and tubular pattern. All grades and stages of tumors were represented. Peripheral blood from each patient was collected in EDTA as a normal control. Macroscopically pure tumor was dissected from the frozen biopsies and leukocytes were pelleted from blood samples before extraction and purification of DNA (25).

PCR Amplification. DNA from tumor and venous blood was analyzed for LOH by amplification of dinucleotide repeat-containing sequences using PCR and the conditions described previously (21). The markers used in this study are shown in Fig. 1. All primer sequences are available from Research Genetics (Huntsville, AL) or the Genome Database (Johns Hopkins University, Baltimore, MD).

Comparative Multiplex PCR. Multiplex PCR was performed essentially as described (21) with the modification that where possible a noninformative locus in an area of hemizygous loss was chosen as a control marker. In this case, approximately twice as much tumor DNA compared to normal DNA is loaded onto the gel so that the control marker is of equal intensity in the normal and tumor DNA lanes. Any significant decrease in signal intensity of the locus of interest in the tumor lane should indicate homozygous deletion. The relative diminution of signal is dependent on the level of normal cell contamination of the tumor.

Southern Analysis and DNA Probes. Restriction digestion of genomic DNAs, blotting, labelling of the probe fragment, hybridization, and stripping of the filter were carried out as described previously (21). A 760-base-pair EcoRI-Xbal fragment from pfulFNa (IFNa) and a 4.2-kilobase BamHI fragment from pEF1D62 (DSP) were used to sequentially probe MspI-digested DNA. A 350-base-pair CDKN2/p16 exon 1 PCR product and a 3.75-kilobase PstI fragment from pMHZ1O (DSP11) were used to sequentially probe EcoRI digests.

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3 The abbreviations used are: RCC, renal cell carcinoma; LOH, loss of heterozygosity; CDKN2, cyclin-dependent kinase 4 inhibitor/p16/MTS1.

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PCR Amplification, Cloning, and Sequencing of CDKN2/p16. Exons 1 and 2 of the CDKN2/p16 gene were separately amplified using the primer sequences and conditions described (23) with the modification that UDP ends were added to the 5' end of each primer for rapid cloning into a CloneAmp vector (GIBCO-BRL, Gaithersburg, MD). Cloned products were used to transform competent DH5-α bacteria and approximately 200 pooled clones were sequenced en masse with the same amplification primers as described (16).

Results and Discussion

Paired normal and primary tumor DNAs from 42 patients were screened for LOH with 9 microsatellite markers on chromosome 9p and an additional 9 markers on 9q (Fig. 1). Fourteen of 42 (33%) tumors showed partial or entire deletion of chromosome 9. Ten of the 14 tumors showed LOH at every informative marker, indicating monosomy of chromosome 9 (Fig. 2a). Two tumors (RCC23 and 28) revealed loss of the p arm only. One tumor (RCC15) showed deletion of the q arm only and an additional tumor (RCC14) had loss of distal q only (Fig. 1). A similar level of loss was observed in the 38 tumors of clear cell type regardless of histology (tubular or papillary pattern). There was also no difference in LOH when the tumors were analyzed for grade and stage. The frequency of chromosome 9 loss rivals that reported previously for any chromosome other than 3p in renal cell carcinoma (4, 10, 11).

The deletion-mapping data suggest that there is a suppressor locus involved in renal tumorigenesis located on both the long and short arms of chromosome 9. A high percentage of monosomy of chromosome 9 was also described in bladder cancer (13) and subsequently, distinct tumor suppressor loci were localized to each arm in bladder cancer (26) and other neoplasms (14). The relative contribution of each putative tumor suppressor genes on p and q is not possible to determine since monosomy of chromosome 9 may target either locus or both. Although one previous report (4) found lower levels of chromosome 9 loss in renal cell carcinomas, the single marker used to assess 9p (D9S15) is now known to map to proximal 9q (27). The high percentage of LOH described by us is based on analysis of many more polymorphic microsatellite markers and therefore informative tumors.

One of the 2 tumors in our study with LOH confined to the q arm showed loss at GSN (which maps to q33) but retained heterozygosity at all proximal informative loci (Fig. 2b). This patient was uninformative at all other loci tested (D9S162 and D9S171) with apparent retention of heterozygosity (homozygous deletion) at IFNa in tumor 25. The IFNa signal in the tumor lane appears disproportionately strong because of the logarithmic amplification of the PCR. N, normal DNA; T, tumor DNA.

Fig. 1. Deletion map of chromosome 9. Markers used and map positions are illustrated on the left. Solid bar on the right, critical region of 9p21. IFNa and D9S736 are less than 500 kilobases apart.

Fig. 2. Microsatellite analysis of primary renal cell carcinomas. (a) Tumor 27 showing monosomy of chromosome 9 with loss of the lower allele at D9S157 and of the upper allele at D9S126 and D9S53. (b) Tumor 14 shows selective deletion of 9q. Retention of heterozygosity is evident at D9S176 and HXB. Loss of the upper allele is seen at GSN. (c) Loss of the upper allele at D9S162 and D9S171 with apparent retention of heterozygosity (homozygous deletion) at IFNa in tumor 25. The IFNa signal in the tumor lane appears disproportionately strong because of the logarithmic amplification of the PCR. N, normal DNA; T, tumor DNA.
DELETIONS OF CHROMOSOME 9 IN RENAL CELL CANCER

(a) RCC9

(b) RCC25

IFNα

D9S7

Fig. 3. Southern analysis of Mspl digested normal (N) and tumor (T) DNA. (a) Hemizygous deletion with the nonpolyomorphic IFNa probe and loss of the lower allele at D9S7 in tumor 9. (b) Homozygous deletion of IFNa with loss of the upper allele at D9S7 in tumor 25. The same filter was probed with IFNa and then stripped and reprobed with EFDB26.3 (D9S7), which maps to distal 9q34, to act as a control for loading differences between lanes. Although the retained D9S7 allele in the tumor lane of b is slightly underloaded compared to the lower D9S7 allele in the normal lane, the predominant IFNa constant band is almost absent in the tumor lane compared to the normal lane. Arrows, alleles.

(21). This tumor was informative for the 2 closest flanking markers to IFNa, D9S162, and D9S171, both of which lost heterozygosity (Fig. 2c). Deletion mapping on 9p therefore delineated a critical homozygously deleted region between D9S171 and D9S162 at 9p21–22 (Fig. 1). To confirm the presence of this homozygous deletion we used a comparative multiplex PCR assay with primers for both IFNa (target) and D9S146 (control). The test revealed that when the D9S146 alleles (s) in the normal and tumor DNA lanes were of equal intensity, the signal for IFNa in the tumor lane was clearly diminished. The IFNa locus in RCC25 is therefore homozygously deleted and not retained; the residual signal from the deleted alleles in the tumor lane arises from normal cell contamination of the tumor biopsy. This is the first report of selective deletions of the p arm and homozygous deletion of 9p21–22 in primary renal cell carcinoma.

Prior studies have shown the region of 9p21–22 surrounding the IFNa locus to be homozygously deleted in many tumor types (17–22). A candidate gene, CDKN2/p16, has recently been identified on 9p21–22 and found to be homozygously deleted in various tumor cell lines including 5 of 9 kidney tumor cell lines (23, 24). To investigate the potential inactivation of CDKN2/p16, the primary tumors with q deletions (and RCC25) were excluded and the remaining 11 tumors with hemizygous deletions through 9p21 were screened for point mutation by sequence analysis of exons 1 and 2 of CDKN2/p16. As noted previously, we found no mutations in these primary renal cell carcinomas (29). However, if homozygous deletion is the predominant mechanism of inactivation then undetected small homozygous deletions around CDKN2/p16, not extending to the nearest markers IFNa, D9S736, and D9S171, could still be missed. In order to exclude this possibility, additional multiplex PCR and Southern analysis were performed to screen for homozygous deletion of CDKN2/p16. Exon 2 of CDKN2/p16 was amplified together with a control marker and performed to screen for homozygous deletion of CDKN2/p16. Exon 2 of CDKN2/p16 was amplified together with a control marker and analyzed as described (21); no additional homozygous deletions were observed. Normal and tumor DNAs were then digested with either EcoR1 or Nspl and probed by Southern analysis with exon 1 of CDKN2/p16 and IFNa (Fig. 3). Southern analysis confirmed homozygous deletion of IFNa extending into CDKN2/p16 in one tumor, RCC25; but no additional homozygous deletions or rearrangements were evident at CDKN2/p16 in the other tumors.

These carefully selected and prepared primary renal cell carcinomas consistently displayed clear allelic losses (Figs. 2 and 3). Therefore, the lack of frequent homozygous deletion or point mutation of CDKN2/p16 in primary renal cell carcinoma suggests that inactivation of CDKN2/p16 occurs by another mechanism or that another gene at 9p21 is the target of the observed deletions. Because abundant point mutations of CDKN2/p16 have been described in familial melanoma, esophageal, and pancreatic cancer (30–32), we favor the latter hypothesis. Further studies aimed at isolating and characterizing putative tumor suppressor genes on chromosome 9 will clarify the critical targets of loss in the progression of renal cell carcinoma and many other neoplasms.

References


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