Deletion of Chromosome 3p14.2-p25 Involving the VHL and FHIT Genes in Conventional Renal Cell Carcinoma

Farkas Sükös, Naoto Kuroda, Tamas Beoto, Amrit Pal Kaur, and Gyula Kovacs

Laboratory of Molecular Oncology, Department of Urology, Ruprecht-Karls-University, D-69120 Heidelberg, Germany [F. S., N. K., A. P. K., G. K.]; Department of Pathology, University of Szeged, 6701 Szeged, Hungary [F. S.]; and Department of Urology, University of Pecs, 7643 Pecs, Hungary [T. B.]

ABSTRACT

Loss of heterozygosity (LOH) at chromosome 3p and inactivation of the VHL gene are associated with the development of conventional renal cell carcinomas (RCCs). Recently, it was suggested that LOH at the FHIT gene at 3p14.2 is an early event in the development of RCC and is characteristic for all types of RCC. We have analyzed 30 papillary, and 22 chromophobe RCCs for LOH at the VHL and FHIT loci and at other loci on chromosome 3p. A continuous deletion of 3p14.2-p25 harboring the VHL and FHIT genes occurred in 96% of the conventional RCCs but only in 10% of the papillary RCCs and 18% of the chromophobe RCCs. Our data indicate that LOH at chromosome 3p14.2-p25 is specific for conventional RCC and that loss of one allele of both the VHL and FHIT genes occurs in early stage of tumorigenesis.

INTRODUCTION

Deletion of chromosome 3p is the most frequent genetic change in sporadic and VHL disease-associated conventional RCCs, whereas alteration of this region is rarely seen in papillary and chromophobe RCCs (1). Comprehensive chromosomal and DNA studies detected allelic loss at chromosome 3p in 96–100% of the series of conventional RCCs, indicating that this genetic change is a crucial event in tumor development (2, 3). A germ-line mutation of the VHL gene at chromosome 3p25 is associated with the development of conventional RCCs (4). Inactivation of the VHL gene by mutation or methylation, however, occurs in only 60–65% of the sporadic conventional RCCs, suggesting the existence of another tumor suppressor gene (5, 6). Deletion mapping and chromosomal replacement studies have identified several nonoverlapping regions along the entire chromosome 3p (for review, see Ref. 3). Although some genes were cloned from these regions, none of them has been proved to be a tumor suppressor gene (7–10). The fragile histidine triad (FHIT) gene at the most common fragile site FRA3B at chromosome 3p14.2 has also been suggested to be involved in the genetics of RCCs (7). Recently, Velickovic et al. (11, 12) reported that LOH occurs selectively at the FHIT gene and suggested that LOH at the FHIT locus is an early event in the development of “clear” cell RCC. Moreover, they suggested that LOH at chromosome 3p is a universal phenomenon in all “morphotypes” of kidney cancer. These data are in strong disagreement with our previous chromosomal and LOH studies. To reconcile this contradiction, we have also analyzed the three major types of RCCs for allelic changes at chromosome 3p including the VHL and FHIT regions.

MATERIALS AND METHODS

Samples and DNA Extraction. Fresh tumor and normal kidney parenchymal tissues were obtained by nephrectomy at the Departments of Urology, Hannover Medical School and Heidelberg University, Germany between 1986 and 1988 and between 1993 and 1997, respectively. We have included 11 small conventional RCCs obtained from a patient with VHL disease. This study comprised 43, 15, 36, and 2 conventional RCCs of pathological stage T1–2, T3a, T3b, and T4, respectively. Grade 1, 2 and 3 disease was identified in 38, 38, and 20 cases, respectively. The size of conventional RCCs varied between 0.4 and 14 cm. Six tumors were smaller than 1 cm, four tumors displayed a size between 1 and 2 cm, and seven tumors were between 2 and 3 cm. Altogether, 67 conventional RCCs were smaller than 7 cm in diameter. Thus, all stages of tumor development are represented in this series of conventional RCCs. The experiment was approved by the Ethical Committee of the Heidelberg University.

A piece of tumor was immediately processed for short-term cultures. Another piece of tumor and corresponding normal kidney tissue were immediately snap-frozen in liquid nitrogen and stored at −80°C, whereas the remaining tissue was fixed in 4% buffered formaldehyde for histological report. The diagnosis was established according to the Heidelberg Classification (13). Based on the histological analysis, the type of some of the renal cell tumors cannot be determined with complete certainty. Therefore, to establish the diagnosis, we have previously analyzed the chromosomal regions specifically involved in the genetics of conventional, papillary, and chromophobe RCCs each by two or three microsatellites (14–18).

Primary cell cultures were established by a combined collagenase and mechanical treatment of tumor tissues as described previously (2). Cells from primary cultures or first passages containing exclusively tumor cells were trypsinized and pelleted. In 6 of the 96 conventional RCCs, 7 of the 30 papillary RCCs, and all cases of chromophobe RCCs, only frozen tissues were available. A frozen tumor sample was placed in a plastic Petri dish, covered with 2 ml of TE9 buffer, and allowed to thaw. The tumor cells were then carefully scraped or pushed out to separate them from stromal tissue under an inverted microscope by a pathologist (G. K.) experienced in this technique. The stromal tissue rests were then discarded. Tumor cells from cell culture or tissues afterward were resuspended in 3–5 ml of TE9 buffer with 1% SDS and 0.2 mg/ml proteinase K and incubated for 5 h at 55°C. DNA was extracted by phenol-chloroform and dissolved in TE buffer after ethanol precipitation.

Normal control DNA was extracted from corresponding kidney parenchymal specimens by the same method. The concentration of DNA samples was adjusted to 50 ng/μl.

Microsatellite Analysis. Microsatellite markers and their approximate positions are shown in Fig. 1. The sequences and location of the markers were obtained from the Genome Sequencing Project.5 The FHIT gene is mapped between 492 and 758 kb within the sequence segment NT_005607.11, whereas the loci D3S1540, D3S1234, and D3S1300 are mapped to 346, 865, and 1268 kb, respectively. The VHL gene is located between 10.648 and 10.661 kb within the sequence segment NT_005978.11, whereas the flanking microsatellite loci D3S1597, D3S1317, and D3S1038 are assigned to 9.831, 10.669, and 10.978 kb, respectively. Matched normal and tumor DNA samples were amplified in 10 μl reactions with 50 ng of genomic DNA, 50 μM KCl, 10 mM Tris-HCl (pH 8.3), 1.5 mM MgCl2, 200 μM each deoxynucleoside triphosphate, 5 pmol of Cy5-labeled forward primer, 5 pmol of reverse primer, and 0.5 unit of Taq DNA polymerase (Life Technologies, Inc., Eggenstein, Germany). After 2 min of denaturation at 94°C, the PCR mixes were subjected to the following conditions: 40 s at 94°C, 30 s at 55°C, and 40 s at 72°C for 28 cycles followed by a step for 10 min at 72°C in a PTC200 thermal cycler (MJ

Received 7/22/02; accepted 11/13/02.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

5 Both authors contributed equally to this work.

To whom requests for reprints should be addressed, at Laboratory of Molecular Oncology, Department of Urology, Ruprecht-Karls-University, Im Neuenheimer Feld 325, Room 002, D-69120 Heidelberg, Germany. Phone: 49-6221-566519; Fax: 49-6221-564634; E-mail: gyula.kovacs@urz.uni-heidelberg.de.

The abbreviations used are: VHL, von Hippel-Lindau; RCC, renal cell carcinoma; LOH, loss of heterozygosity; AI, allelic imbalance.
scored when the signal at one allele was decreased or increased to around of intensity of one allele in tumor cells decreased to zero or nearly zero. AI was according to our scoring system (19). Briefly, LOH was scored when the signal normal DNA. Examples are shown in Fig. 1. Allelic changes were evaluated whereas DNA prepared from tumor tissues by our method contained analysis, DNA from short-term culture of tumor cells was free of normal DNA, software (Amersham/Pharmacia Biotech). According to the microsatellite

C.

°

Fig. 1. LOH at locus D3S1597 in two conventional RCCs. DNA was isolated from short-term culture in case 536 and from primary tissue in case 172. Note the complete loss of signal at allele 2 in case 536 and a weak signal at allele 2 in comparison with normal allele in case 172.

Research Inc., Watertown, MA). Before loading, 20 μl of stop solution containing 50 mM EDTA and 5 mg/ml Dextran Blue 2000 in 100% deionized formamide were added, and the samples were denatured at 95°C for 2 min. Analysis was carried out on an automated DNA sequencer (ALFExpressII; Amersham Pharmacia Biotech, Freiburg, Germany). The 6% denaturing polyacrylamide gels were run at 400 V, 55 mA, 30 W in 1× Tris-borate EDTA buffer at a constant gel temperature of 55°C.

The collected data were evaluated using the Fragment Manager (FM 1.2) software (Amersham/Pharmacia Biotech). According to the microsatellite analysis, DNA from short-term culture of tumor cells was free of normal DNA, whereas DNA prepared from tumor tissues by our method contained <10% normal DNA. Examples are shown in Fig. 1. Allelic changes were evaluated according to our scoring system (19). Briefly, LOH was scored when the signal intensity of one allele in tumor cells decreased to zero or nearly zero. AI was scored when the signal at one allele was decreased or increased to around of 50% of the allelic signal seen in normal cells.

RESULTS

Conventional RCC. We have detected LOH at chromosome 3p in 92 of 96 conventional RCCs (Fig. 2). LOH at the loci D3S1038 and D3S1317, which are located 817 kb distal from and 8 kb proximal to the VHL gene, occurred in all informative cases. LOH at locus D3S1540, which is located 46 kb proximal to the FHIT gene, and at locus D3S1234, which is mapped 107 kb distal, has also been detected in all but one informative case. LOH at all informative loci between the VHL and FHIT genes was also seen in all but the one exceptional case, where the breakpoint occurred between loci D3S1283 and D3S1559. Excluding this case, the smallest overlapping region of LOH was determined between loci D3S1038 and D3S1234, both included. The distal breakpoint was identified between D3S1038 and 3pier, whereas the proximal breakpoint is located between D3S1234 and the centromere. In summary, a large continuous region of chromosome 3p14.2-p25 including one allele of the VHL and FHIT genes was deleted in 91 of the 96 conventional RCCs. Each of the six conventional RCCs between 4 and 9 mm in size from a VHL patient showed a breakpoint proximal to the FHIT gene, and the large deletion included the VHL gene as well.

Papillary RCC. An AI at all informative loci was seen in 2 of the 30 papillary RCCs. One of them was analyzed previously by karyotyping and showed a trisomy of chromosome 3. In a third tumor, we found LOH along the short arm of chromosome 3. This case was also analyzed previously by karyotyping and showed a trisomy of chromosome 8 as a sole abnormality (20). The clear cut LOH in this tumor can be explained by a recombination event involving the entire chromosome 3p. Thus, allelic changes at chromosome 3p occurred in 10% of papillary RCCs.

Chromophobe RCC. LOH at all informative loci was seen in 3 of 22 chromophobe RCCs. In a fourth case, LOH was seen at locus D3S1286 and at each locus located distally, whereas heterozygosity was retained by all proximal loci (Fig. 1). LOH at chromosome 3p occurred in 18% of chromophobe RCCs.

DISCUSSION

We detected allelic changes at chromosome 3p at high frequency in conventional RCCs (96%) and at low frequency in papillary RCCs (10%) and chromophobe RCCs (18%). There is a tendency toward an extreme chromosomal loss in chromophobe RCCs, which may explain a random loss of chromosome 3 in some cases (1, 15). Duplication of chromosome 3q, which is associated with the progression of papillary RCCs, may appear in some cases as a trisomy of chromosome 3 (20). Such chromosomal duplications can be detected as an AI (2:1) when analyzed by microsatellites (16). Thus, our data, together with previous observations on distinct types of RCC, strongly suggest that LOH at chromosome 3p is a specific genetic alteration in conventional RCCs. In contrast to our data, Velickovic et al. (11, 12) found LOH at chromosome 3p only in 76% of the “clear cell” RCCs but in 86% of chromophobe RCCs and 59% of the papillary RCCs by analysis of a series of RCCs in archival material and therefore suggested the LOH at 3p as a universal phenomenon in all RCC “morphotypes.”

A “zebra” pattern of loss of chromosome 3p in RCCs has been described by several investigators during the last 15 years (for review, see Ref. 3). Recently, Alimov et al. (21) found four regions of interest analyzed previously by microsatellites markers and the deletions in the 88 conventional (cRCC), 30 papillary (pRCC), and 22 chromophobe (chrRCC) renal cell tumors. Vertical lines represent the chromosome 3p segment deleted in RCCs, whereas the numbers at the top indicate the cases with deletions.
including the VHL but not the FHIT locus. Velickovic et al. (11) detected LOH occurring selectively at the FHIT gene and suggested the principal role of this gene in the development of conventional RCCs. Remarkably, they found LOH at locus D3S1300 flanking the FHIT gene in 43% of the conventional RCCs and LOH at locus D3S1038 flanking the VHL gene in only 12% of the conventional RCCs. Analyzing RCC cells from short-term cultures, we detected LOH at locus D3S1300 as well as at locus D3S1038 in 95% of conventional RCCs. Taking into account that LOH occurred at each locus between the VHL and FHIT genes in all but 1 of the 92 conventional RCCs, our microsatellite study indicates a continuous region of deletion at chromosome 3p14.2-p25. Therefore, a "conventional RCCs, our microsatellite study indicates a continuous tumor suppressor gene (7–10). Because a zebra" pattern of LOH at chromosome 3p in RCC should be evaluated as a technical artifact, and the interstitial deletions suggested by these studies may not be the right regions for positional cloning.

The deletion of a large chromosome 3p21.3-p25 region occurred in 92 of 96 conventional RCCs including tumors of 4–9 mm in size. This observation indicates that loss of one allele of both the VHL and FHIT genes occurs at an early stage of tumorigenesis. Clinicopathological and experimental studies suggest that inactivation of the VHL gene plays a critical role in the development of conventional RCC, but the involvement of FHIT in the genetics of RCC remains controversial (4–6, 22, 23). None of the other genes cloned from the 3p14.2-p25 region proved to be a classical tumor suppressor gene (7–10). Because the 3p14.2–25 region is deleted in nearly all conventional RCCs, few tumors remain to be searched for a tumor suppressor gene locus. Hopefully, analyzing such exceptional cases by a bacterial artificial chromosome array covering the whole chromosome 3p will uncover a small specific deletion and allow the positional cloning of the RCC gene.

REFERENCES
Deletion of Chromosome 3p14.2-p25 Involving the VHL and FHIT Genes in Conventional Renal Cell Carcinoma

Farkas Sükös, Naoto Kuroda, Tamas Beothe, et al.


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/63/2/455

Cited articles
This article cites 23 articles, 8 of which you can access for free at:
http://cancerres.aacrjournals.org/content/63/2/455.full.html#ref-list-1

Citing articles
This article has been cited by 4 HighWire-hosted articles. Access the articles at:
/content/63/2/455.full.html#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.