Allelic Loss at the Predisposing Gene Locus in Spontaneous and Chemically Induced Renal Cell Carcinomas in the Eker Rat

Yoshiaki Kubo, Hiroaki Mitani, and Okio Hino

Department of Experimental Pathology, Cancer Institute, 1-37-1 Kami-ikebukuro, Toshima-ku, Tokyo 170, Japan

ABSTRACT

Hereditary renal carcinoma (RC) in the rat, originally reported by Eker in 1954, is an example of a Mendelian dominant predisposition to a specific cancer in an experimental animal. We previously reported that ionizing radiation induces additional tumors in a linear dose-response relationship, suggesting that in heterozygotes two events (one inherited, one somatic) are necessary to produce tumors. Recently, the predisposing gene has been mapped to rat chromosome 10. This study was designed to examine loss of heterozygosity (LOH) at chromosome 10 in the RCs developed from hybrid F1 rats carrying Eker mutation. In spontaneous RCs, 6 of 10 (60%) showed loss of the wild-type allele covering over 30 cm, consistent with two-hit hypothesis. Individual tumors have different patterns of LOH even from the same kidney, showing independent clonal origins of RCs. In contrast, none of N-ethyl-N-nitrosourea-induced RCs had allelic loss (0 of 9, P < 0.01). Thus, the nature of the second event differs between spontaneous and chemically induced tumors in the Eker rat. These results suggest that chemically induced tumors in experimental animals involve intragenic mutations and so do not cause LOH of syntenic markers. Interestingly, 1 of 5 spontaneous pituitary tumors that developed in the Eker rat showed LOH for chromosome 10 markers.

INTRODUCTION

A class of cancer genes, called tumor suppressor genes or anti-oncogenes, has been revealed by the study of hereditary human cancers (1). Although these genes are recessive in oncogenesis, they render carriers heterozygous and so appear in pedigrees, as dominantly inherited disorders. Such a dominantly inherited predisposition was described in the rat by Eker and Mossige (2). The heterozygous Eker rat typically develops RCs through multiple stages, from early preneoplastic lesions (e.g., atypical tubules) to adenomas, and penetrance for this RC is generally complete (3).

We previously reported that: (a) germ line homozygosity for the Eker mutation is lethal at around 10 days of fetal life (3); (b) according to our radiation experiment, renal tumors in heterozygotes appear to involve two hits, one of which is inherited in the Eker rat and the other of which can be induced by a somatic event (3); (c) the predisposing gene of the Eker rat has been mapped to the proximal part of chromosome 10 (4), which we subsequently confirmed (5); (d) rat chromosome 10 currently has no syntenic relationship to human chromosome 3p (6), the presumed site of the putative tumor suppressor gene responsible for human RC (7, 8) and of the locus of von Hippel-Lindau disease, which predisposes to multiple RCs (9); (e) a putative tumor suppressor gene near the interferon locus on rat chromosome 5 seems to be important in tumor progression, but is not the RC gene (3); (f) the Eker rat is highly susceptible to induction of RCs, not Wilms' tumor, by transplacental administration of ENU (10). Recently, Yeung et al. (4) found LOH for rat chromosome 10 markers in 4 of 5 spontaneous renal tumors of hybrid F1 offspring (LE/Fisher 344 strains), supporting the recessive tumor suppressor nature of the predisposing gene. In the present study, we examined chemically induced renal tumors in F1 rats carrying the Eker mutation for LOH, and compared the results with those obtained in spontaneous tumors.

MATERIALS AND METHODS

Animals. Founder rats (two males and three females) carrying the Eker mutation were kindly provided by Dr. Alfred G. Knudson (Fox Chase Cancer Center, Philadelphia, PA). Eker rats were bred on a LE background (Charles River Breeding Laboratory, USA) by brother × sister mating and were maintained pathogen free in the Animal Facility of Cancer Institute since 1991. All animals were housed and treated in accordance with institutional guidelines. Eker rats were diagnosed as carriers by detecting microscopic kidney tumors following unilateral nephrectomies around 6 months of age.

Experimental Procedure. Two male rats carrying the Eker mutation (RC/+, LE strain of Charles River Breeding Laboratory, USA) were mated with several normal female rats (+/+) of the LE strain (Kiwa Breeding Laboratory, Japan) and inbred BN strain (Charles River Japan, Inc.) to produce hybrid F1 rats showing DNA polymorphisms. Even in the same LE strain, there are DNA polymorphisms between LE (USA) and LE (Japan) strains. For the ENU experiment, a normal female rat (+/+, LE strain of Kiwa Breeding Laboratory; 8 weeks old), which had been mated with one male rat carrying the Eker mutation (RC/+, LE strain of Charles River Breeding Laboratory, USA), was exposed to a single carcinogenic dose of ENU (80 mg/kg of body weight, i.p.; Nalcalai Tesque Inc., Kyoto, Japan) on the 15th day of gestation as previously reported (10). Pregnancy was confirmed by demonstration of sperm in the vaginal smears. The day when sperm were noted was designated as gestation day 0.

DNA Samples. We analyzed 10 spontaneous renal tumors from 7 rats [75 weeks, 77 weeks, 81 weeks, 86 weeks (LE/BN), 88 weeks, 90 weeks, and 99 weeks old], and 9 ENU-induced renal tumors from 4 rats (25 weeks, 27 weeks, 31 weeks, and 38 weeks old). Fresh samples of renal tumors and normal liver were obtained from carrier rats (RC/+), and were immediately exposed to X-ray film with an intensifying screen at —70°C for 1—2 days. HiScreen DNA Samples. We analyzed 10 spontaneous renal tumors from 7 rats [75 weeks, 77 weeks, 81 weeks, 86 weeks (LE/BN), 88 weeks, 90 weeks, and 99 weeks old], and 9 ENU-induced renal tumors from 4 rats (25 weeks, 27 weeks, 31 weeks, and 38 weeks old). Fresh samples of renal tumors and normal liver were obtained from carrier rats (RC/+), and were immediately frozen at —80°C. High molecular weight genomic DNAs were extracted by digestion with 2% SDS/proteinase K followed by extraction with phenol as reported (11).

Histology. Histology of the removed kidneys were examined macroscopically for histology. Tissues were fixed in 10% formalin and a routine histological examination was carried out on hematoxylin and eosin-stained paraffin sections.

Southern Blot Analysis. DNAs (10 μg/lane) were digested with restriction enzymes showing polymorphisms between LE (USA) and LE (Japan)/BN strains, electrophoresed on a 1% agarose gel, and transferred onto nylon membranes (Biodyne, Pall Biosupport) in 0.4 M NaOH. As previously reported (5), these blots were hybridized overnight at 65°C in a solution containing 0.2 M NaHPO4 (pH 7.2), 1 mM EDTA (pH 8.0), 1% bovine serum albumin, 7% SDS, and 32P-labeled DNA probes (1—3 X 106 cpm/ml) prepared by a random oligonucleotide-priming procedure using a Hybristributor (Model HR-1, Nippon Genetics). The filters were washed twice in 1 X standard saline citrate, 0.1% SDS at room temperature for 15 min each, and at 65°C for 30 min. They were exposed to X-ray film with an intensifying screen at —70°C for 1—2 days.

Polymorphic DNA Probes. The rat neurofibromatosis type 1 (NF1) gene (gift of Dr. H. Saya, The University of Texas M. D. Anderson Cancer Center), mouse RFP-1 gene (gift of Dr. T. Taniguchi, Osaka University), and the mouse protamine-1 (PRM-1) gene (gift of Dr. R. Yeung, Fox Chase Cancer Center; originated from the American Type Culture Collection), all of which map to rat...
allelic W5S in Eker rat renal tumors were used as polymorphic DNA markers.

PCR. The oligonucleotide primers of rat interleukin 3 (5'-CTGCTTA-GAGCTTACACACA-3' and 5'-AGGAATTCTGTCAGTTTACT-3') (5, 12) were made by a DNA synthesizer (Milligen/Biosearch, Division of Millipore). PCR was performed (5) in 25 µl of a reaction mixture containing 100 ng of the genomic DNA, 1 pmol/µl of the primers, 1 mM MgCl₂, 200 µM concentration each of dGTP, dATP, dTTP, and dCTP, and 2 units of Taq polymerase (Biotech). Thirty-five cycles of amplification, each consisting of denaturation for 60 s at 92°C, annealing for 60 s at 55°C, and extension for 90 s at 72°C were performed with a thermal programmer (QT II or QTPI; Nippon Genetics). The products of the PCR were electrophoresed on 1.5% low melting point agarose gels (Gibco BRL) and visualized with ethidium bromide.

Definition of LOH. The signal intensity of the polymorphic alleles was quantified by a Hoefer GS-300 scanning densitometer; peak areas corresponding to each signal were calculated by electric integration with the use of the GS-370 1-D electrophoresis data system (Hoefer Scientific Instruments, San Francisco, CA). The intensities of signals in tumor tissue DNAs were compared to those of normal DNAs. When 50% reduction in signal intensity was detected, it was judged as LOH, as reported by Fujiwara et al. (13).

RESULTS AND DISCUSSION

The inheritance of a RC mutation determines the specificity of tumor histology even with in utero carcinogenesis; i.e., adult type renal cell carcinoma, but not Wilms’ tumor (10). Thus, the ENU-induced and spontaneous renal tumors found in this study were all RCs. No recognizable histological differences were observed between the two groups, although ENU-induced RCs develop much earlier than do spontaneous RCs (e.g., 30 ± 6 weeks old versus 85 ± 8 weeks old in this study, respectively). The spontaneous RCs in the Eker rat develop slowly over the course of 10–12 months before reaching macroscopic size, while it takes only 3 months to reach the same size in ENU-induced RCs (10). In the control rats (noncarriers), no RCs developed in this experimental system. In the Eker rat inheritance of a RC mutation reduces the required number of events. Under conditions in which ENU produces no tumors in controls it produces RC in the Eker rat. According to linkage analysis, the predisposing gene is mapped to the same locus in both spontaneous and ENU-induced RCs in the back-cross animals (4, 5).

NFI, IRF-1, and PRM-1 genes clearly showed polymorphisms among LE (USA)/LE (Japan) and BN strains with Taq 1, Taq 1/BamH1 and EcoRI (Figs. 1 and 2), respectively. NFI did not show polymorphism between LE (USA) and LE (Japan). PCR products of the IL-3 locus also showed polymorphism among LE (USA)/LE (Japan) and BN strains (Fig. 3). In Eker rat, not only RCs, but also other tumors, develop (14, 15). Interestingly, 1 of 5 spontaneous pituitary tumors that we found in the present study had LOH at interleukin 3 locus (Fig. 3).

LOH on chromosome 10 was clearly detected in spontaneous RCs, as shown in Figs. 1 and 3, and the wild-type allele was always lost, consistent with the two-hit hypothesis. We excluded macroscopically "normal" parts from tumor tissues as much as possible. It was noted that the loss of the wild-type allele can be detected in primary tumors.
even when using a highly sensitive PCR assay, although in some tumors a weak band of the wild-type allele was observed due to normal cellular component (e.g., stromal cells, endothelium, WBC, etc.) (Fig. 3). Our data basically support the previous report (4). The results are summarized in Fig. 4 (6 of 10, 60%). Among tumors from the same rat, the patterns of LOH are different (Fig. 4), showing their independent clonal origins rather than metastatic ones. In contrast, none of the 9 RCs (0%) induced by ENU showed LOH (Figs. 2, 3, and 4). This is a statistically significant difference (P < 0.01) in rates (6 of 10 = 60% versus 0 of 9 = 0%). ENU is a well-characterized mutagen and carcinogen that acts by direct ethylation of the bases of DNA.

REFERENCES

Allelic Loss at the Predisposing Gene Locus in Spontaneous and Chemically Induced Renal Cell Carcinomas in the Eker Rat

Yoshiaki Kubo, Hiroaki Mitani and Okio Hino


Updated version

Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/54/10/2633

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.