Proc. Natl. Acad. Sci. USA
Vol. 85, pp. 1571–1575, March 1988
Genetics

Consistent chromosome 3p deletion and loss of heterozygosity in renal cell carcinoma

(carcinogenesis/deletion mapping)

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Contributed by George Klein, October 9, 1987

ABSTRACT **Renal cell carcinoma (RCC) and normal kidney tissues have been examined from 34 patients with sporadic, nonhereditary RCC. Eighteen of the 21 cytogenetically examined tumors (86%) had a detectable anomaly of chromosome arm 3p distal to band 3p11.2–p13, manifested as a deletion, combined with the nonreciprocal translocation of a segment from another chromosome or monosomy 3. Restriction-fragment-length polymorphism analysis showed loss of *D1S1* heterozygosity in 16 of the 21 cases (76%). *D3S2* heterozygosity was lost in 2 of 11 cases (18%). The variability of the breakpoint between 3p11.2 and 3p13 and the absence of a consistently translocated segment from another chromosome suggests a genetic-loss mechanism, while the activation of a dominant oncogene appears less likely. Together with the previously demonstrated involvement of the 3p14.2 region in a familial case, these findings suggest that RCCs may arise by the deletion of a "recessive cancer gene," as do retinoblastoma and Wilms tumor. The relevant locus must be located on the telomeric side of the *D1S1* locus on the short arm of chromosome 3.**

Renal cell carcinoma (RCC) is the most common adult form of malignancy in the human kidney. The disease occurs during the sixth and seventh decades of life and is usually sporadic but also can occur in a hereditary form (1–3) characterized by an earlier age of onset and the frequent appearance of multiple primary tumors. The hereditary form is often bilateral, while the sporadic form is unilateral.

Cytogenetic studies identified the short arm of chromosome 3 as the most frequent site of nonrandom aberrations. Cohen *et al.* (1) described a large Italian-American family with a constitutional 3;8 translocation. Five translocation-carrying members of the family who reached the age of 37 years or more developed primary RCC, while 12 translocation-free members who reached a corresponding age did not (1). The translocation breakpoint was localized to 3p14.2 (4). Another RCC family has been described by Pathak *et al.* (2). Its members did not carry any constitutional cytogenetic aberration, but the cells of the single cytogenetically examined tumor had a 3;11 translocation. The breakpoint was in the same region (3p13–p14) as the constitutional translocation breakpoint in the family described by Cohen *et al.* (1).

Cytogenetic analysis of sporadic RCC also has revealed chromosome 3 anomalies. Wang *et al.* (5) have detected deletions and unbalanced translocations affecting the short arm of chromosome 3 in RCC biopsies and derived cell lines. Yoshida *et al.* (6) found similar 3p-associated deletions and translocations in 8 of 12 nonfamilial cases of RCC. Carroll *et al.* (7) have identified the 3p12–p21 region as the site of deletions or translocations in five of six clear cell tumors. Kovacs *et al.* (8) have found 3p anomalies in 22 of 25 sporadic RCCs, with a breakpoint cluster in the 3p11.2–p13 region.

Chromosomal changes can contribute to the tumorigenic process in at least two different ways. Translocations may displace a dominant oncogene into a highly active chromosome region, with constitutive activation as the result. Alternatively, they may damage or delete "recessive cancer genes" that can trigger tumor development by their loss. The first category can be exemplified by the juxtaposition of c-*myc* to immunoglobulin gene sequences in certain B-cell-derived rodent and human tumors (for a review, see ref. 9); the second can be exemplified by the abnormal BCR-ABL fusion protein in Philadelphia chromosome (Ph^1)-positive chronic myelogenous leukemia (10). The loss or functional inactivation of both alleles of a single gene plays an essential role in the development of retinoblastoma, Wilms tumor, and some other solid tumors (11–17).

To assess the occurrence and possible significance of the 3p deletion in RCC, we have examined 34 tumors by a combination of cytogenetic and molecular methods, focusing on the short arm of chromosome 3.

MATERIALS AND METHODS

Tumors, Cell Cultures, and Chromosome Analysis. A total of 34 RCC patients from Hannover Medical School and Siloah Hospital were studied. Tumors were obtained from untreated patients by radical nephrectomy. Cytogenetic analysis was performed as described (8). DNA isolation restriction enzyme digestion, agarose gel electrophoresis, Southern transfer, blot hybridization, and quantitative densitometric scanning of autoradiograms were performed as described (18, 19).

RESULTS

Chromosome 3p Aberrations. Of the 34 RCCs, 21 were studied cytogenetically (Table 1). Eight of them have been reported in an earlier paper by Kovacs *et al.* (8). Of the 21 tumors, 18 contained an abnormal chromosome 3, while 3 (HA221, HA229, and HA235) did not. All 18 had lost the distal part of the short arm of chromosome 3 by unbalanced chromosomal translocations or monosomy of chromosome 3 (Table 1). The shortest-region overlap analysis localized a consistent change to the 3p13–pter region. The deleted segment of the short arm of chromosome 3 was not detectable elsewhere in any of the karyotypes. Different segments of other chromosomes, derived most frequently from chromosome 5, were transposed to the breakpoint on chromo-

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Abbreviations: RCC, renal cell carcinoma; RFLP, restriction-fragment-length polymorphism.

Table 1. Loss of heterozygosity on chromosome 3 in RCC

Cases	Aberrations of chromosome 3	Allelic assignments with 3p gene probes			
		D1S1	*D3S2*	*D3S3*	*D3S6*
		NK TU	NK TU	NK TU	NK TU
HA184	ND	12: 2	1 :	2:	1 :
HA187	der(3)t(3;5)(p13;q22)	1 :	12:12	2:	1 :
HA188*	der(3)t(3;5)(p13;q11.2)	2:	1 :	12:12	1 :
HA200	ND	12: 2	1 :	2:	1 :
HA218	ND	2:	12:12	2:	1 :
HA219	ND	12:1	1 :	2:	1 :
HA221*	None visible	12:12	1 :	2:	1 :
HA229	None visible	12:12	1 :	12:12	12:12
HA231	ND	12: 2	12:12	ND	1 :
HA232	ND	12: 2	1 :	2:	1 :
HA235	None visible	2:	1 :	2:	12:12
HA266	−3	12:1	1 :	2:	1 :
HA267*	der(3)t(3;5)(p13;q22)	12:1	ND	ND	1 :
HA271*	der(3)t(3;5)(p13;q11.2)	12:1	12:12	2:	1 :
HA275	ND	12: 2	1 :	2:	1 :
HA277	ND	12:12	1 :	12:12	1 :
HA279	ND	2:	1 :	12:12	1 :
HA284*	der(3)t(1;3)(q11;p13)	12:12	12:12	2:	1 :
HA295	der(3)t(3;5)(p13;q22)	2:	12:1	2:	1 :
HA306	der(3)t(3;?)(p13;?)	2:	12:12	ND	ND
HA312*	der(12)t(3;12)(p11.2;q24)	12:1	1 :	1 :	1 :
HA316	der(3)t(3;?)(p13;?)	12:1	12:12	2:	ND
HA320*	der(3)t(3;5)(p13;q22)	1 :	12: 2	2:	1 :
HA322	der(3)t(3;?)(p13;?)	12: 2	2:	12:12	1 :
HA344	der(?)t(3;?)(q12;?)	2:	12:12	2:	1 :
HA345	der(17)t(3;17)(q12;q25)	12:1	1 :	2:	1 :
HA349*	der(3)t(3;7)(p12.2;q11)	1 :	2:	12:12	12:12
HA350	ND	1 :	1 :	12:12	1 :
HA356	ND	12: 2	2:	2:	1 :
HA357	ND	2:	1 :	12:12	2:
HA364	ND	12:12	2:	2:	1 :
HA366	der(3)t(3;5)(p13;q22)	12: 2	ND	2:	1 :
HA386	der(3)t(3;?)(p13;?)	12:1	12:12	2:	1 :
HA387	der(3)t(3;?)(p13;?)	1 :	1 :	12:12	1 :

Allelic assignments were determined by Southern hybridization to 3p gene probes. For each probe, alleles are indicated with 1 as the larger restriction fragment and 2 as the smaller fragment. Polymorphic probes used in the Southern hybridization are indicated at the top of the table. In certain cases the resultant autoradiogram was analyzed by scanning densitometer, and peak areas corresponding to each hybridization signal were calculated by electronic integration. According to densitometric analysis, in HA231, the intensity of allele 1 decreased by 45%; in HA322, by 75%; in HA356, by 20%; and in HA366 by 30%. The intensity of allele 2 decreased in HA271 by 70%; in HA312, by 30%; in HA316, by 20%; and in HA386, by 80%. ND, not determined.
*Reported by Kovacs *et al.* (8).

some 3. The 3p breakpoint could be localized to band p13 in 13 of the 21 cytogenetically analyzed tumors (Table 1). The rearrangement of chromosome 3 was the only karyotypic change in 2 RCCs (HA306 and HA366); 6 other tumors (HA187, HA267, HA312, HA320, HA322, and HA344) had only one or two other chromosomal changes in addition to the chromosome 3 aberration. The remaining 10 tumors had a few additional chromosomal anomalies, without any discernible pattern (data not shown). No chromosomal aberrations were found in normal kidney cells karyotyped by G-banding.

Loss of Chromosome 3-Associated Restriction-Fragment-Length Polymorphism (RFLP) Heterozygosity. Parallel studies were performed to detect the loss of heterozygous RFLP markers, mapped to the short arm of chromosome 3 (Fig. 1). DNA was prepared from 34 primary RCC and corresponding normal kidney tissues of the same patient. *Hin*dIII or *Msp* I restriction enzyme-digested DNA fragments were separated by agarose gel electrophoresis and analyzed by Southern blot hybridization.

Four chromosome 3p-specific recombinant DNA probes were used (Fig. 1). *D1S1* originates from chromosome 3 but contains a 1.7-kilobase (kb)-long *Pst* I–*Hin*dIII repetitive element that is also present on chromosome 1. *Hin*dIII-cleaved human DNA yields four bands hybridizing to the *D1S1*-specific H3H2 clone. The 2.3- and/or 2.0-kb fragments define the RFLP locus mapped to 3p21 (21, 22). The *D3S2* probe has been mapped to 3p14–3p21 (20, 23, 24). *Msp* I digestion of human DNA results in two polymorphic fragments, 2.9 and/or 1.3 kb in size (23, 24). The *D3S3* probe detects a polymorphic locus at 3p14 detected as a 4.8-kb single band and/or two bands of 3.6 kb and 1.2 kb after *Msp* I digestion (25, 26). *D3S6* has been localized to the broad region from q21–pter on chromosome 3 and has been detected as 1.4- and/or 1.0-kb bands upon *Msp* I digestion of the DNA (27).

Normal kidney DNA was heterozygous for *D1S1* in 21 of 34 cases. *D3S2* heterozygosity was present in 11 cases; *D3S3*, in 9 cases; and *D3S6*, in 3 cases (Table 1). Eight patients were heterozygous for two RFLPs, and 1 was heterozygous for three loci (Table 1).

Eight tumor DNAs derived from *D1S1* heterozygotes had lost one of the two polymorphic bands. Fig. 2 shows a representative analysis of the loss of heterozygosity in the

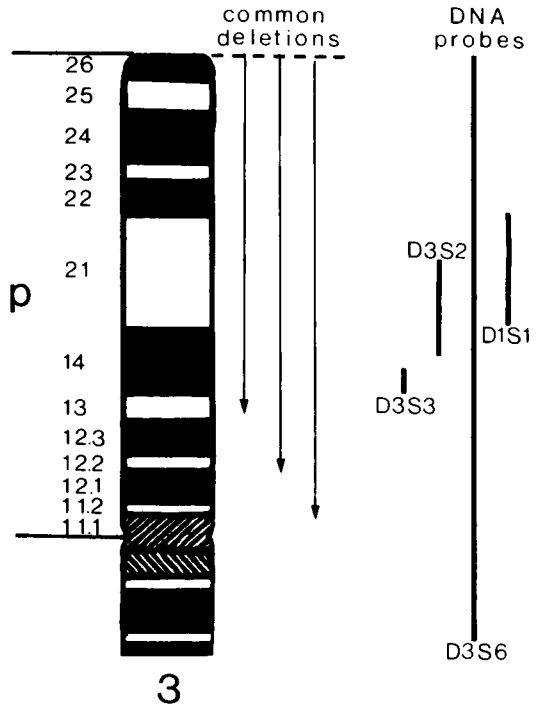


FIG. 1. Normal chromosome 3, showing the conventional regional assignments of RFLP loci (20–27). The localization of *D1S1* is probably more proximal (see text). The common breakpoints are designated by arrowheads.

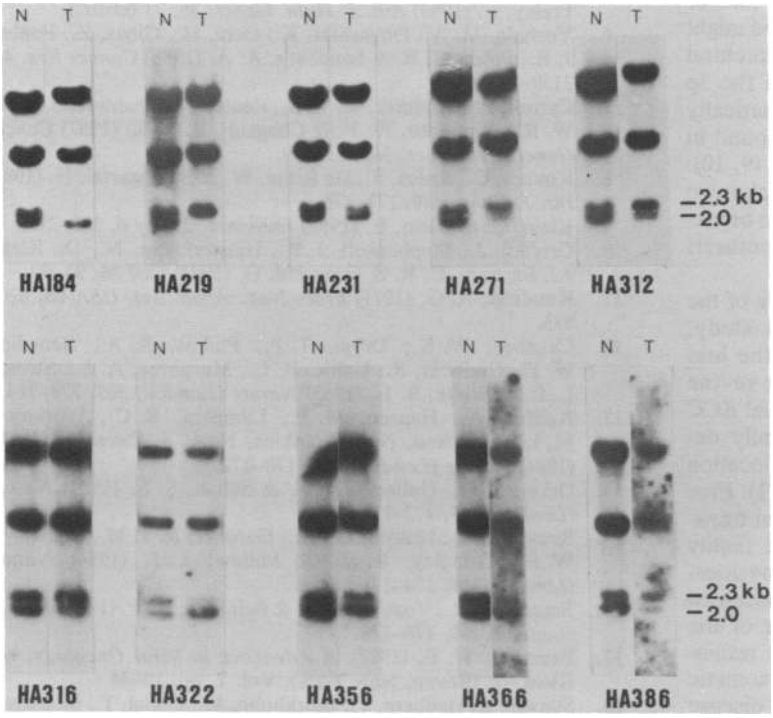
tumor cell DNAs. There was no complete allelic loss in eight tumors, but the densitometric analysis showed a significant reduction in the intensity of one allele in the autoradiograms (Fig. 2 *Left* and Table 1). Contaminating nontumor cells may be responsible for the remaining weak hybridization signal. Heterogeneity of the tumor cells was less likely because the chromosomal aberration was present in the entire clonal tumor cell population. Two of the 11 *D3S2* heterozygotes showed a tumor-associated loss of one RFLP locus (Table 1 and Fig. 2 *Right*).

Among seven tumors with identical breakpoints at 3p13, HA267, HA271, HA316, HA322, HA366, and HA386 had lost *D1S1* heterozygosity, while HA284 had maintained it (Fig. 3 and Table 1). The same phenomenon has been observed for the *D3S2* locus: HA187, HA271, HA284, HA295, HA306, HA316, HA320, and HA386 had the same breakpoint at 3p13, but only HA295 and HA320 were homozygous for *D3S2* (Table 1). Similarly, the tumor HA188 with the chromosomal breakpoint at 3p13 maintained *D3S3* heterozygosity (Fig. 3 and Table 1). Nine patients were heterozygous for two or more RFLP loci. Five of them, HA231, HA271, HA316, HA322, and HA386 had lost *D1S1* but maintained *D3S2* or *D3S3* heterozygosity (Table 1). The chromosomal breakpoint was at 3p13 in four cytogenetically analyzed tumors (HA271, HA316, HA322, and HA386).

DISCUSSION

We have found that the short arm of chromosome 3 was lost in 18 of 21 RCCs, in agreement with earlier studies (5–7). Previously, Kovacs *et al.* (8) have shown that chromosomal breaks are clustered at bands p13, p12.2, and p11.2; 3p13 represents the most common breakpoint. Segments from other chromosomes have been translocated to the deleted short arm of chromosome 3 in 14 cases. The breakpoints on chromosome 3 were clustered in the same region (3p11.2–3p13) as in the simple deletions (8). The long arm of chromosome 5 served as a donor of the translocated piece in 8 RCC tumors (8). Eighteen of the 21 (86%) tumors studied cytogenetically in the present investigation have lost the 3p14–pter region.

The 3p21-specific *D1S1* sequences were lost in 16 of 21 (76%) informative patients, while the *D3S2* sequences, mapped to the 3p14.2–p21 region (20, 23, 24) were lost in only 2 of 11 (18%). This implies that the most frequent breakpoint is probably quite close to *D1S1* on the proximal side and distal to the *D3S2* locus. The mapping of the *D1S1* locus to the telomeric side of *D3S2* and *D3S3* is particularly clear in the tumors HA271 and HA316. The fact that RCCs with the same cytogenetic breakpoint (3p13) could either lose or maintain *D1S1* heterozygosity suggests that the *D1S1*



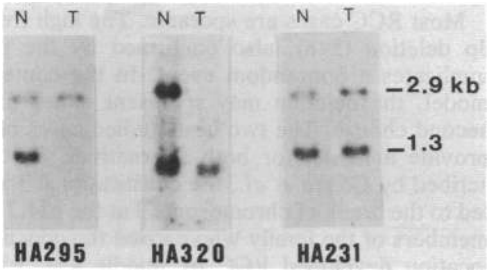


FIG. 2. Representative Southern hybridization of 3p probes to normal kidney tissue (lanes N) and RCC (lanes T) DNA. The patient designation is under the blots. (*Left*) *Hin*dIII digest, probe pH3H2 (*D1S1*). (*Right*) *Msp* I digest, probe p12-32 (*D3S2*).

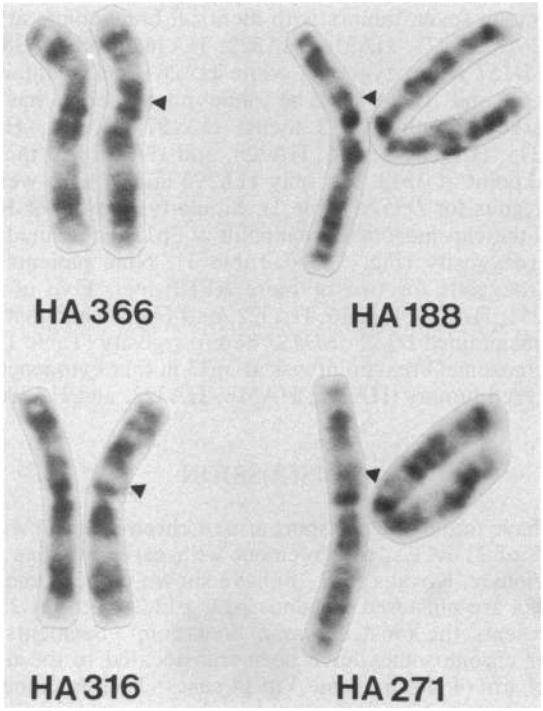


FIG. 3. Partial karyotypes showing the normal (*Left*) and the rearranged (*Right*) chromosome 3 of patients HA366, HA188, HA271, and HA316. The arrowheads show the breakpoints at 3p13 on the rearranged chromosomes.

sequence has a more proximal location than its previously mapped position at band p21. Our findings also indicate that the locus involved in the genesis of RCC is on the telomeric side of the *D1S1* locus. *D3S3* and *D3S6* must be localized on the proximal side of the breakpoint.

The chromosome 3p deletion may contribute to the development of RCC in one of two basically different ways: activation of a dominant oncogene or loss of a recessive cancer gene. The oncogene activation hypothesis would imply that a dominantly acting protooncogene is localized at the breakpoint. Removal of regulatory sequences and/or invisible translocation of a gene from a different region might be responsible for the constitutive activation or structural change of the relevant gene. The wide variation of the 3p breakpoints speaks against this possibility. Cytogenetically recognizable breakpoint variations have not been found in the Ig/MYC or *BCR/ABL* translocation prototypes (9, 10). The cytogenetically invisible translocation of an unknown segment from some other chromosome to the deletion breakpoint cannot be definitely excluded, but the loss hypothesis appears more likely.

Most RCC cases are sporadic. The high frequency of the 3p deletion (5–8), also confirmed by the present study, implicates a nonrandom event. In the context of the loss model, the deletion may represent either the first or the second change. The two best-studied cases of familial RCC provide a model for both alternatives. In the family described by Cohen *et al.*, the constitutional 3;8 translocation led to the break of chromosome 3 at the p14.2 band (1). Five members of the family who carried the constitutional translocation developed RCC by middle age, while 12 family members who did not receive the constitutional translocation and reached comparable age did not. It may be assumed that the second locus was inactivated by any one of the possible cytogenetic mechanisms that can act in retinoblastoma. Since this would have to occur during the somatic history of the kidney, the long latency period of the disease may relate to the low rate of cell divisions or, alternatively, to the intervention of other tumor-promoting cellular or microenvironmental changes. It may be noted that the familial tumors appeared at the median age of 45 years, one decade before the peak incidence of sporadic RCC. In another RCC family, Pathak *et al.* (2) have identified a 3;11 translocation that was restricted to the tumor. It may be speculated that the cytogenetically invisible deletion may have affected the first locus in this case, while the somatic translocation break has damaged the second. It may be hypothesized that the break involved in the constitutive 3;8 translocation of Cohen *et al.* (1) and the somatic 3;11 translocation of Pathak *et al.* (2) localize the critically important gene to the p13–14.2 region. It is then likely that the cytogenetic and RFLP deletions detected in our present study act by the loss of the same gene. In that case, the breakpoints on the short arm of chromosome 3 must lie between the postulated p13–14.2 site of the gene and the centromere and can never be distal to that site. This is borne out by our combined cytogenetical and molecular findings.

While this manuscript was prepared, Zbar *et al.* described the loss of *D1S1* or *D3S2* heterozygosity in 11 of 11 sporadic RCCs (28).

We thank Dr. R. White for the *D3S2* and *D3S3* probes, Dr. C. H. C. M. Buys for *D1S1*, and Dr. K.-H. Grzeschik for *D3S6*. Drs. G. W. Bornkamm and G. Scherer are acknowledged for initial discussions. This work was supported by National Cancer Institute Grant 3R01CA14054, by the Swedish Cancer Society, and by Deutsche Forschungsgemeinschaft (DFG Ko 841/3-1). G.K. is the recipient of a short-term European Molecular Biology Organization Fellowship; R.E., F.B., S.I., and J.S. are recipients of fellowships from the Cancer Research Institute and the Concern Foundation.

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