The most frequently lost allelic site in human renal cell carcinoma (D3F15S2) on the short arm of chromosome 3 has homologous sequences on rat chromosome 8

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Abstract. It has previously been shown that human chromosome 3 has banding homology to rat chromosome 8. We have previously isolated a cDNA from the D3F15S2 region and des-

ignated the gene as RIK. In the present study, we localized the homolog of this gene to rat chromosome 8.

Renal cell carcinoma (RCC) in humans is frequently associated with loss of D3F15S2 (localized to 3p21). We searched the D3F15S2 region for transcribed sequences and were able to isolate a cDNA from a placenta cDNA library (Erlandsson et al., 1990). We designated the gene as RIK.

Somatic rat × mouse hybrids, DNA, and Southern blot hybridization. High-molecular-weight DNA was prepared from a panel of somatic cell hybrid clones derived from the fusion of normal Sprague-Dawley (S-D) rat hepatocytes with the mouse hepatoma line BWTG3. These hybrids are known to segregate rat chromosomes (Szpirer et al., 1984). In the cytogenetic analysis of the rat × mouse hybrid panel used in this work (Szpirer et al., 1984), chromosome 8 could not be identified unambiguously. It is now clear that the difficulties were not only due to the fact that this chromosome resembles mouse chromosomes 9 and 13, but also to the almost systematic absence of this chromosome in these hybrids (Szpirer et al., 1988b).

Twenty micrograms of high-molecular-weight DNA were completely digested with *Bam*HI under the conditions recommended by the manufacturer (Amersham International, Arlington Heights, Illinois). Conditions for probe labeling, DNA digestion, filter hybridization, and film exposure were as described (Kovacs et al., 1988).

Probe. The probe used was C14-2, a 2.3-kb human cDNA fragment of RIK (Erlandsson et al., 1990). It was ³²P-labeled by the standard "oligolabeling" method to a specific activity of 5 × 10⁸ cpm/μg.

Chromosome localization in rat. To localize the rat RIK gene, we screened *Bam*HI-digested rat × mouse hybrids with the human cDNA probe. The 9-kb and 4-kb rat RIK-specific bands

Fig. 1. Hybridization of a RIK-specific probe to a Southern blot of *Bam*HI-digested DNA from rat × mouse hybrid cell lines and controls. Lane 1, mouse control, Balb/c spleen; lane 2, rat control, S-D spleen; lane 3, negative, LB330; lane 4, positive, LB810. The rat-specific germ line bands are at 9 and 4 kb, and the mouse-specific band is at 14 kb.

were present in LB810 but not in the other 15 hybrid lines tested. Figure 1 gives examples of positive and negative hybrids. Comparison with the chromosomal segregation data (Table I) shows that only rat chromosome 8 gave a consistently concordant pattern, indicating that the rat RIK locus is on chromosome 8.

Detection of the RIK gene at stringent hybridization conditions in rat, mouse, hamster, and human genomes demonstrated that the gene is highly conserved. This implies that the gene has a household function.

It has been reported that the D3F15S2 homolog in mouse is localized to chromosome 9 (Lalley et al., 1989). We have confirmed that finding (data not shown). Mapping of the RIK locus to mouse chromosome 9 and rat chromosome 8 are in agreement with the detection of conserved banding homology between rat and mouse chromosomes (Szpirer et al., 1988a).

Supported by the Swedish Cancer Society, the Erik Philip-Sörensen Foundation, the Trigger Foundation, The Inga-Britt och Arne Lundbergs Forskningsstiftelse, National Institutes of Health grant 5 R01CA14054, the CGER-ASLK (Belgium), and the Belgian Ministère de Politique Scientifique (Science de la Vie). R.E. received stipends from The Syskonen Svensson Fund, the Robert Lundbergs Minnesfond, and the Swedish Cancer Society. F.B. and S.I. are recipients of fellowships from the Swedish Cancer Society. F.B. is a recipient of a fellowship from the Cancer Research Institute and Cancer Foundation.

Received 6 December 1990; revision accepted 1 April 1991.

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Table I. Correlation of rat-specific RIK sequences with rat chromosomes in rat × mouse somatic cell hybrids

Hybridization/ chromosome	Rat chromosomes																				
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	X
+/+	0	1	1	1	0	1	1	1	0	1	1	1	1	1	1	1	1	0	1	1	1
-/-	12	8	10	4	11	8	7	15	13	9	10	8	4	10	9	4	9	3	10	12	3
+/-	1	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0
-/+	3	7	5	11	4	7	8	0	2	6	5	7	11	5	6	11	6	12	5	3	12
Discordant hybrids	4	7	5	11	5	7	8	0	3	6	5	7	11	5	6	11	6	13	5	3	12

The number of hybrids that are concordant (+/+ or -/-) or discordant (+/- or -/+) with the rat RIK sequence are given for each chromosome.

The rat RIK gene is syntenic with the TF and PCCB genes on chromosome 8 (Szipirer et al., 1988b, 1989). These three genes are also syntenic in man on chromosome 3 (3p21, 3q21→q26, and 3q13.3→q22, respectively) (Yang et al., 1984; Kraus et al., 1986). The RIK, TF, and PCCB genes thus define a syntenic group that is conserved in rat and man.

Losses affecting the short arm of human chromosome 3 are associated with a number of neoplasias, eg., small cell lung carcinoma and renal cell carcinoma. Renal carcinomas and other

solid tumors in rats and mice might be profitably scored for corresponding losses on rat chromosome 8 and mouse chromosome 9, respectively.

Note added in proof. Since the localization of the RIK gene to rat chromosome 8 and mouse chromosome 9, we have found that it codes for acylpeptide hydrolase (Erlandsson R, Boldog F, Persson B, Zabarovsky ER, Allikmets RL, Sümegi J, Klein G, Jörnvall H: The gene from the short arm of human chromosome 3, at D3F15S2, frequently deleted in renal cell carcinoma, encodes acylpeptide hydrolase. *Oncogene* 6:1293–1295, 1991).

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