Brief Communication

Rat c-raf Oncogene Is Located on Chromosome 4 and May Be Activated by Sequences from Chromosome 13

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Received 11 August 1987—Final 10 March 1988

Abstract—Activated forms of the protooncogene c-raf have been found to transform established lines of rodent fibroblasts after transfection with DNA from several human and rat tumors. Using Southern blot analysis of DNAs from rat × mouse somatic cell hybrids, we have mapped c-raf to rat chromosome 4. An exogenous sequence that was found juxtaposed to c-raf within transforming DNA originally derived from a rat hepatocellular carcinoma was localized to chromosome 13.

INTRODUCTION

v-raf was identified as an oncogene of a transforming murine retrovirus, 3611-MSV (1). Little is known about the physiological function of raf, except that v-raf protein fused with gag has serine–threonine kinase activity in vitro (2). Rearranged and activated cellular homologs of v-raf have been detected by the NIH-3T3 transfection assay in a human gastric adenocarcinoma (3); human breast, lung, and renal carcinomas (4); a human glioblastoma maintained in nude mice (5); and chemically induced rat hepatocellular carcinomas (6). Similar rearrangements were found in the different human tumor DNAs and in the rat hepatocellular DNA, affecting the regions in or around exons 6–9. Activation of c-raf was attributed to truncation of the amino-terminal sequence and possibly to elevated expression at the transcriptional (4, 7) or translational level (8), due to an attached foreign DNA sequence. No rearrangement could be detected in the raf loci of the original tumors, suggesting that the rearrangements occurred in the course of the transfection experiment in vitro or were only present in a small subpopulation of the original tumor (4, 7, 8). The v-raf and the artificially activated c-raf are truncated in a similar way, resulting in a gene that contains only the 3' portion of the normal cellular gene (9).

Following the transfection of NIH-3T3 cells with DNA from the rat hepatocellular carcinoma (6), the activated c-raf gene produced a fused mRNA containing an unknown rat sequence that had replaced the 5' end of the c-raf message (8). The rearranged, but not the normal, c-raf gene could transform NIH-3T3 cells when linked to a retroviral LTR (8).

In view of the potential significance of the raf gene for tumor development and the increasing evidence that chromosomal rearrangements may activate oncogenes, we have determined the rat chromosomal localization
of c-raf and of the sequence that has replaced the 5' half of the gene in the experiment with hepatocellular carcinoma (8, 10). c-raf was mapped to chromosome 4 and the transposed sequence to chromosome 13, suggesting that c-raf activation in the hepatocellular carcinoma-derived DNA was due to the recombination of genetic material derived from chromosomes 4 and 13.

MATERIALS AND METHODS

Rat × Mouse Hybrids. 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 (11). The chromosomal constitution of the hybrid clones is shown in Table 1.

Southern Blot Analysis and Probes. Twenty micrograms of high-molecular-weight DNA were completely digested with EcoRI under the conditions recommended by the manufacturer (Amersham International, Arlington Heights, Illinois). The fragments were separated in a 0.6% agarose gel and transferred to a nylon filter. HindIII-cleaved lambda phage DNA was used as a size marker. The probes were a 1.6-kb human liver cDNA fragment containing c-raf-1 sequences (12), inserted into the HindIII-EcoRI sites of pSP65 by the Amersham laboratories (Code RPN.1319X), and a 0.7-kb EcoRI–HindIII fragment from pA5, the 5' part of the activated c-raf (8). The probes were 32P-labeled by the “oligolabeling” method (13) to a specific activity of \( 5 \times 10^8 \) cpm/\( \mu \)g and hybridized to the filters at a concentration of \( 3 \times 10^6 \) cpm/ml. The c-raf-1 filters were washed in 1 × standard sodium citrate (SSC), 0.1% sodium dodecyl sulfate (SDS) at 60°C, and the pA5 filters in 0.2 × SSC, 0.1% SDS at 65°C, and autoradiographed.

RESULTS

To localize the rat c-raf gene, we screened EcoRI-digested rat × mouse hybrid panels with a human c-raf probe (12). The 12-kb and 7-kb EcoRI rat c-raf specific bands were present in LB161, LB251, LB330, LB330TG2, LB330TG3, LB330TG6, LB510, LB600, LB630, LB780, LB810, LB860, LB1040, LB1040TG3, and LB1040TG5 but not in the other three hybrid lines tested, LB20, LB210D, and LB210I. To localize the sequence transposed to c-raf during transfection with rat hepatocellular carcinoma-derived DNA, we screened the same hybrid panel with the pA5 probe (8). The 16-kb EcoRI rat pA5 specific bands were present in LB20, LB161, LB210B, LB210C, LB210D, LB210I, LB251, LB510, LB600, LB630, LB780, LB810, and LB860, but not in the other seven hybrid lines tested, LB330, LB330TG2, LB330TG3, LB330TG6, LB1040, LB1040TG3, and LB1040TG5. Figure 1 gives examples of positive and negative hybrids. Comparison with the chromosomal segregation data (Table 1) shows that only rat chromosomes 4 and 13 gave a consistently concordant pattern, indicating that the rat c-raf and pA5 loci are on chromosomes 4 and 13, respectively.

DISCUSSION

We have mapped the rat c-raf locus to chromosome 4. This chromosome shows a homologous banding pattern with mouse chromosome 6 (14, 15), known to carry the murine c-raf gene (16). The immunoglobulin kappa chain (IgK) (17) and the Ki-ras-2 gene (18) are also localized on rat chromosome 4 and mouse chromosome 6. Our mapping of c-raf to rat chromosome 4 is consistent with an extensive synteny between the two chromosomes. The corresponding human genes are located on three different chromosomes [IGK