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The N-*ras* oncogene assigned to the short arm of human chromosome 1

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ABSTRACT

The human N-ras oncogene, isolated from the HL-60 promyelocytic leukemia cell line, is distantly related to viral oncogenes of Kirsten and Harvey sarcoma viruses. We have determined its chromosomal location by Southern blot analysis of DNAs from 37 human x rodent hybrid cell lines derived from 8 different human donors, some of whom carried balanced rearrangements of chromosome 1. The results indicate that the N-ras oncogene (RASN) is localized on the proximal part of the short arm of human chromosome 1, in region p3200→cen.

INTRODUCTION

Cellular proto-oncogenes (c-onc genes) are DNA sequences homologous to the transforming genes of retroviruses and to active transforming sequences isolated from several human tumor cell lines (for review see 2). The transforming sequences of at least five different types of human tumors, detected by DNA mediated gene transfer into NIH 3T3 mouse fibroblasts, are homologous to different sequences of the same family of retrovirus oncogenes: the ras family. The transforming sequences of the human bladder carcinoma cell lines EJ and T24 are homologous to the v-Ha-ras gene, while those from certain lung and colon carcinoma cell lines are homologous to the v-Ki-ras gene (3-8). Transforming sequences isolated from neuroblastoma cell line SK-N-SH (9) and from the human HL-60 promyelocytic cell line (1,10) have been shown to be closely related to each other and probably also to sequences derived from a human fibrosarcoma and a rhabdomyosarcoma cell line (1). This new transforming gene has not been identified with any known retroviral oncogene but appears to be partially homologous to the oncogenes of Harvey and Kirsten sarcoma viruses (1). It is therefore considered to be a member of the same gene family and has been named the N-ras oncogene (1).

Knowledge of the chromosomal localization of the normal cellular counterparts of oncogenes may be important in further understanding the

mechanisms leading to activation of the oncogenes. This information may also shed light on the molecular basis of karyotypic alterations present in certain tumors (11-13). At least eleven c-onc genes have already been localized (12-21) and among them, homologues of viral Harvey and Kirsten ras oncogenes on human chromosomes X, 6, 11 and 12 (22-25). In this study we investigated the chromosomal localization of the most recently characterized member of this family, the N-ras oncogene. By means of Southern analysis of somatic cell hybrids, we localized the N-ras gene on the proximal part of the short arm of human chromosome 1.

MATERIALS AND METHODS

Hybrid cell lines. We analyzed 37 different rodent x human hybrid cell lines segregating human chromosomes. They were derived from 8 independent series of fusion experiments, using three different rodent cell lines. 380-6 cells are hypoxanthine phosphoribosyl transferase deficient derivatives of Chinese hamster lung fibroblast line V79; they were fused with six different normal diploid human donor cell strains to give rise to series X (26), XII and XIII (27), XV (28), XVII (29) and XVIII hybrids (Francke et al, unpublished). A-23 cells are thymidine kinase deficient derivatives of Chinese hamster Don cells and were the rodent donor of series XXI hybrids (30,31). Series XIX hybrids were constructed from a fusion between rat hepatoma cells 7777-14b-aza with a human skin fibroblast strain (32). After expansion in culture all hybrid cell lines were recharacterized by chromosome and isozyme analysis as previously described (27,28,31,33). For each series, the number of hybrid cell lines analyzed in this study is given in parentheses: series X (1), series XII (4), series XIII (5), series XV (5), series XVII (1), series XVIII (10), series XIX (4), and series XXI (7).

Southern blot analysis. High molecular weight DNAs were extracted from the hybrid and the parental cell lines, digested to completion with EcoRI (New England Biolab), transferred to nitrocellulose filters (Schleicher and Schuell BA 85) and hybridized to ^{32}P-labelled probe, as described (33), with the following modifications: hybridization was carried out at 68°C for 14 to 20 hours, in a buffer containing .75 M sodium chloride, .075 M sodium citrate, but without formamide. Filters were ultimately washed in .015 M sodium chloride .0015 M sodium citrate at 68° C.

The plasmid p52C$^-$ derived from pUC8, carries a subcloned 1.1 kbp PvuII fragment, isolated from a bacteriophage obtained from a genomic library. This library was constructed from the DNA of NIH 3T3 cells transformed by the

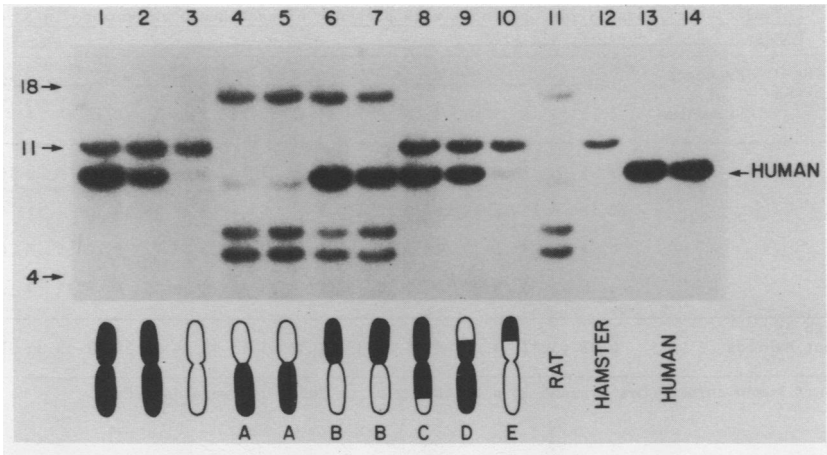

Figure 1. Regional mapping of N-ras on chromosome 1. EcoRI digested DNA (10 µg/lane) from rodent x human somatic cell hybrids (lanes 1-10) and controls (lanes 11-14) were probed with plasmid p52C⁻. Hybrids in lanes 1, 2, 3, 8, 9 and 10 were Chinese hamster x human. Hybrids in lanes 4, 5, 6, and 7 were rat x human. Diagrams of human chromosome 1 indicate the region present in black and the region absent in white for each hybrid sample.

human N-ras oncogene (1). The plasmid insert has been shown to derive from within the N-ras oncogene of the HL-60 promyelocytic leukemia cell line (1). The plasmid was labelled to high specific activity by nick-translation (34) and used as probe for N-ras related sequences.

RESULTS

The HL-60 transforming sequence (N-ras) is localized on human chromosome 1

Initially, a panel of 32 different Chinese hamster x human hybrid cell lines was analyzed. Hybrids were derived from independent fusions between six normal diploid human cell strains and two established Chinese hamster cell lines. Their overlapping chromosome contents were precisely defined, so that any given pattern of hybridization could be correlated with the presence of a specific human chromosome (35). Probe p52C⁻ containing part of the cloned active transforming sequence isolated from the HL-60 cell line (1), was hybridized under stringent conditions to EcoRI digested hybrid and control DNAs on nitrocellulose filters. A single fragment of approximately 7.5-kbp was seen in normal human male and female DNA (Fig 1, lanes 13 and 14). Two Chinese hamster cross-reacting fragments were detected: a strongly hybridizing one of higher molecular weight (approximately 10-kbp) and a weakly hybridizing smaller fragment of about 7.7-kbp (Fig. 1, lane 12). Each

Table 1. Correlation of human N-ras sequences with presence of each human chromosome in 32 Chinese hamster x human somatic cell hybrids

Presence of human 7.5-kbp fragment	Presence of Chromosome	Human Chromosomes																						
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X	Y
+	+	9	6	8	7	6	6	3	9	5	3	8	9	7	11	5	8	4	8	3	6	9	10	8	3
-	-	20	18	8	15	12	11	16	9	18	17	9	12	8	6	13	13	16	13	15	7	11	9	6	5
+	-	0	4	1	3	5	2	8	2	5	8	1	2	4	0	5	3	7	3	8	5	2	0	1	3
-	+	0	3	12	5	9	9	4	9	1	3	11	8	13	12	8	8	5	8	5	14	9	12	12	5
Percentage of discordant hybrids		0	23	45	27	44	38	39	38	21	35	41	32	53	41	42	34	38	34	42	59	35	39	48	50

Only intact human chromosomes present at a frequency of at least .10 were included.

sample was scored for the presence of the human 7.5-kbp fragment. The pattern produced by positive Chinese hamster x human hybrids is illustrated in Fig. 1 lanes 1, 2, 8 and 9, and that of negative Chinese hamster x human hybrids in lanes 3 and 10. Segregation analysis of 32 hybrids revealed that the presence of the human 7.5-kbp fragment correlated with the presence of human chromosome 1 without exception (Table 1). Each of the remaining chromosomes could be excluded by a discordancy rate of at least 20%. This rules out the existence of sequences of comparable homology on any other human chromosome, and localizes the N-ras transforming sequence to human chromosome 1.

Further localization in region spanning from centromere to interface between bands p31 and p32 of chromosome 1.

Eight rodent x human hybrid cell lines containing parts of human chromosome 1 were informative for regional localization. Four independently derived rat x human hybrid lines had undergone spontaneous rearrangements with breaks at the centromere of human chromosome 1. Two of these hybrids contained only the long arm (type A, Fig. 1, lanes 4 and 5) and the other two only the short arm of this chromosome (type B, Fig. 1, lanes 6 and 7) in the absence of the normal chromosome 1 (36). The rearranged chromosome of type C (Fig. 1, lane 8) was derived from a balanced t(1;2)(q32;q13) translocation present in the human donor of hybrid series X (26). Region 1q32→1qter is missing in this hybrid (37). Chromosome types D and E (Fig. 1, lanes 9 and 10) are reciprocal products of the balanced translocation between chromosomes 1 and 6, t(1;6)(p3200; p2100) present in the human donor of hybrid series XV (28). As a result of spontaneous chromosome segregation and repeated subcloning (38), hybrid lines were obtained which contained either one or the

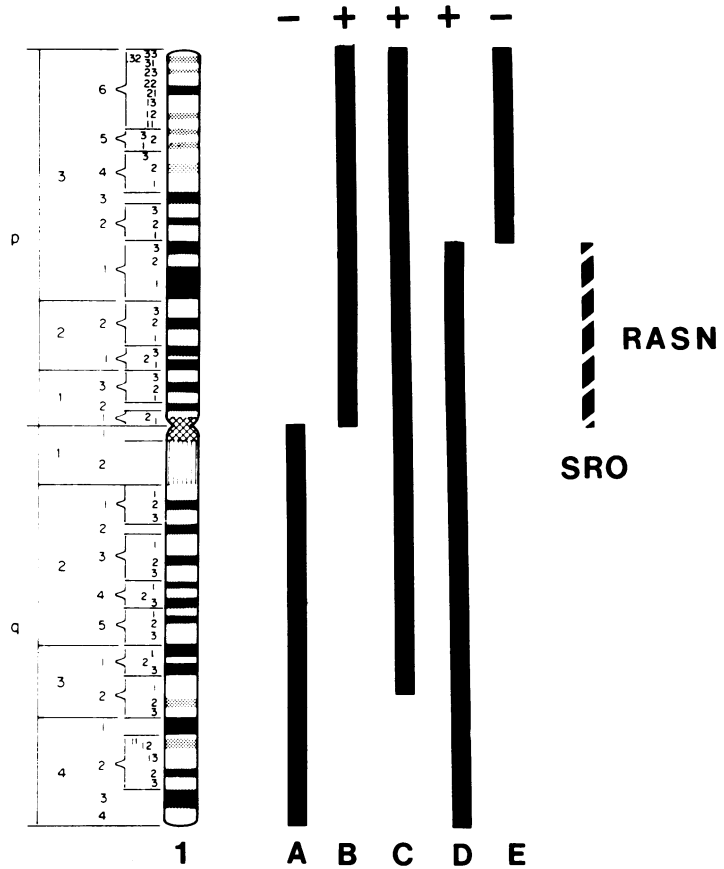

Figure 2. Assignment of N-ras (locus symbol RASN) to the shortest region of overlap (SRO, hatched bar) p3200→cen on chromosome 1. (Ideogram from ref. 52). Bars A through E represent regions present in the different types of hybrids. + and - refer to presence or absence of the human 7.5 kbp EcoRI fragment (Fig. 1).

other derivative chromosome in the absence of the normal homologue. Two independent hybrids with chromosome type D were studied and gave identical results.

Filters carrying EcoRI cleaved DNAs from these hybrids and the parental cell lines were incubated with probe p52C⁻ under stringent conditions of hybridization (Fig 1). As mentioned above, there were one 7.5⁻kbp human fragment (lanes 13 and 14), two Chinese hamster fragments (lane 12), and four rat fragments (lane 11). The human fragment, of distinct size, was easily scored in the different samples. In the presence of the long arm alone of

chromosome 1, no sequence homologous to the probe was detected (lanes 4 and 5), while a positive signal was obtained (lanes 6 and 7) in the presence of the short arm alone. However, the distal half of the short arm, as in chromosome type E (lane 10), does not carry the homologous sequences, while the reciprocal translocation product does hybridize (type D, lane 9). This indicates that the N-ras sequence lies in the proximal half of the short arm in the shortest region of overlap between chromosomes type C and D. All results taken together localize the sequences homologous to the HL-60 transforming ras oncogene, called RASN according to recommended human gene nomenclature (39), to the proximal short arm (region p3200→cen) of human chromosome 1 (Fig. 2).

DISCUSSION

Sequences homologous to the HL-60 ras transforming gene were detected by Southern analysis in 14 rodent x human hybrid lines out of 37 studied. At high stringency no human bands of hybridization were seen in the remaining 23 hybrid lines, in particular, not in the presence of human chromosomes 6,11 and 12 to which different ras genes have been assigned (22-25). Any homology between N-ras and these other ras genes can only be detected at reduced stringency of hybridization (1,9). Furthermore, using viral Kirsten and Harvey ras probes for in situ hybridization, Jhanwar and colleagues (24) did not detect a site of specific hybridization on human chromosome 1. Under stringent conditions we found distinct restriction fragments hybridizing strongly with the human probe in DNAs from Chinese hamster and rat parental cell lines indicating evolutionary conservation of N-ras sequences between rodent and human species.

Our results indicate the existence of a single RASN locus on the proximal short arm of chromosome 1, in region 1p3200→cen, i.e. the region spanning from the interface between bands p31 and p32 to the centromere (40)(Fig. 2). Does this localization suggest a particular mechanism of N-ras activation in human tumors? DNA rearrangements and chromosomal translocations of proto-oncogenes represent a possible mechanism of oncogene activation. Well-documented examples are c-myc rearrangements and t(8;14), t(2;8), t(8;22) translocations in Burkitt's lymphoma (12,13,18). In addition, a recent report demonstrates the translocation of c-abl from chromosome 9 to 22 as result of the specific t(9;22) in chronic myelogenous leukemia (14). Human promyelocytic leukemias are often associated with non-random reciprocal t(15;17) translocations (41); the breakpoint on 15

could possibly be related to the site of c-fes (16). In HL-60, however, c-myc sequences located on 8q24 have been amplified (42,43), and the active transforming sequence is N-ras on 1p. The HL-60 karyotype does not reveal a rearrangement of either 1, 8 or 15. In our trypsin-Giemsa banded chromosome analysis of HL-60 cells we found two normal appearing copies of chromosomes 1 and 8. The modal chromosome number was 44 with only one normal chromosome 5, as previously reported by Gallagher et al (44). Consistent structural rearrangements were present that had not previously been detected: a 9p-, 13p+, 16q+ and 17p+. The number of double minutes (DM) varied between 5 and 15 per cell (Francke et al, unpublished data).

Human neuroblastomas, both fresh tumor tissue and established cell lines, often contain cytological correlates of gene amplification, DM or homogeneously staining regions (HSR)(45). The most common structural rearrangements in neuroblastoma involve breaks in the middle of the chromosome 1 short arm (46-48). The breakpoints have been reported between 1p13 and 1p32, with 1p32 and 1p31 most frequently involved (49). It appears tempting to speculate on a relationship between the RASN localization and activation of this oncogene in neuroblastoma, but there is no evidence as yet for such a connection. The human neuroblastoma SK-N-SH which contains an active N-ras transforming sequence (9) has been reported to have no 1p abnormality (49). The mechanism of activation of N-ras in the two tumor cell lines from which the active gene has been isolated does not involve a major chromosome rearrangement at the site of the RASN locus. This does not rule out the possibility that in those neuroblastomas that are associated with a 1p abnormality the N-ras sequences have been altered, translocated elsewhere or amplified within HSRs or DM. At least one neuroblastoma line, IMR-32, has developed an HSR on 1p (48) but N-ras sequences are not amplified in this cell line (9). The sequences amplified within an HSR do not have to come from the same chromosome; amplified regions may arise elsewhere after a translocation (50).

It should be possible to more precisely define the RASN localization using somatic cell hybrids that further dissect the region (51) and in situ hybridization of N-ras probes directly to high-resolution human chromosomes (24). Our data thus far suggest that it may be worthwhile to investigate the organization of N-ras sequences in neuroblastomas and other human tumors with 1p abnormalities.

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