Regional chromosomal localization of *N-ras*, *K-ras-1*, *K-ras-2* and *myb* oncogenes in human cells

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ABSTRACT

The identification of transforming genes in human tumor cells has been made possible by DNA mediated gene transfer techniques. To date, it has been possible to show that most of these transforming genes are activated cellular analogues of the ras oncogene family. To better understand the relationship between these oncogenes and other human genes, we have determined their chromosomal localization by analyzing human rodent somatic cell hybrids with molecularly cloned human proto-oncogene probes. It was possible to assign N-ras to chromosome 1 and regionally localize c-K-ras-1 and c-K-ras-2 to human chromosomes 6pter-q13 and 12q, respectively. These results along with previous studies demonstrate the highly dispersed nature of ras genes in the human genome. Previous reports indicated that the c-myb gene also resides on chromosome 6. It has been possible to sublocalize c-myb to the long arm of chromosome 6 (q15-q21). The non-random aberrations in chromosomes 1, 6 and 12 that occur in certain human tumors suggest possible etiologic involvement of ras and/or myb oncogenes in such tumors.

INTRODUCTION

Highly diverse organisms harbor DNA sequences related to the transforming genes (v-onc) of acutely transforming retroviruses (1). The high degree of conservation of these genes (c-onc) implies their importance in normal eucaryotic cell functions such as growth and/or differentiation. Furthermore, the study of v-onc genes and their cellular homologues has potential importance for elucidating the molecular mechanisms involved in the genesis of human cancers. One approach we have used to gain a better understanding of the interactions of human onc genes and other cellular sequences is chromosome mapping. Our studies and those of others have shown the dispersion of onc genes among several human chromosomes. It has also been possible to show that onc genes reside at or near the points of translocation or deletions in chromosomes which have been found to be specifically altered in certain human tumors such as Burkitt's lymphoma (2-7).

We have utilized somatic cell hybrids constructed between various human cell lines and either mouse or Chinese hamster cells to study the chromosomal

localization, and where possible the regional localization, of members of the ras family of oncogenes. This gene family consists of at least three divergent sets of sequences. c-H-ras, represented by two distinct loci in both rat and human DNA (8, 9), is the cellular homolog of the transforming genes of the Harvey and BALB strains of murine sarcoma viruses (MSV), (v-has, v-bas, respectively) (10). The cellular homolog of the Kirsten MSV transforming gene, designated c-K-ras, also exists in at least two forms in mammalian DNA (9). The v-K-ras nucleotide sequence is only about 80% homologous to v-H-ras (11, 12). A new human ras gene family member, distantly related to either v-has/bas or v-K-ras, has been recently described and is designated N-ras due to its isolation from a neuroblastoma cell line (13). The ras gene family is under intensive investigation since it has been found that the activated forms of three family members (c-H-ras-1, c-K-ras-2, and N-ras) are transforming genes in a wide variety of human tumor cells (14-20). We have previously mapped c-H-ras-1 to human chromosome 11 (21), and this has been independently confirmed by other laboratories (22-25). In our present studies, we confirm the locations of c-K-ras-1, c-K-ras-2, and N-ras to chromosomes 6, 12, and 1, respectively. In addition, we have been able to regionally localize some of these genes. The finding of c-K-ras-1 on chromosome 6, the reported location of the c-myb gene (26), led us to also regionally map c-myb. This oncogene was mapped to the long arm of chromosome 6.

MATERIALS AND METHODS

Cell Culture

Human fibroblast lines used in cell fusions included diploid WI38 (ATCC CCL75), an hprt⁻ simian virus 40-transformed WI18 line (27), a tk⁻ Hela derivative AV3 (ATCC CCL21), and two lines with balanced chromosome translocations (GM 0073 and GM 2658) containing the karyotypes 46X, t(X;14)(Xpter>Xq13::14q32>14qter;14pter>14q32::Xq13>Xqter) and 46,XX,t(2;6)(2pter>2q11::6q15>6qter;6pter>6q15::2q11>2qter), respectively. Rodent lines employed were murine hprt⁻ L-A9 (28), mouse tk⁻ L-B82 (28) and LMTKCLID (29), and an hprt⁻ derivative of the hamster CHV79 line. Human/rodent somatic cell hybrid lines were isolated in selective medium after cell fusion in polyethylene glycol as described (30). Hybrid lines were usually subcloned twice before expansion. The same preparation of cells was used for DNA isolation, isoenzyme analyses, and karyotyping.

Isoenzyme Analyses

Hybrid cell lines and subclones were analyzed for the presence of all

human chromosomes (except Y) by standard isoenzyme analyses (31, 32) using the enzyme markers described (30). These analyses permit identification of the human chromosomes retained in hybrid cells. Specific isoenzyme markers used to identify human chromosomes 1, 6, and 12 were enolase 1 (ENO-1; EC4.2.1.11), phosphoglucosmutases 1 and 3 (PGM-1 & PGM-3; EC2.7.5.1), phosphogluconate dehydrogenase (6-PGD; EC1.1.1.44), peptidases C and B (Pep C & Pep B; EC3.4.11--), fumarase (FH; EC4.2.1.2), soluble malic enzyme (ME-1; EC1.1.1.40), mitochondrial superoxide dismutase (SOD2; EC1.15.1.1), and lactate dehydrogenase B (LDH-B; EC1.1.1.27). Murine and human forms of 6-PGD could not be resolved and human PEP-B, and frequently PEP-C, could not be identified in human/hamster hybrids. Regional chromosomal assignments of these, and other, markers have been described (33).

Molecular Hybridization Probes

Human oncogene clones were isolated from bacteriophage libraries of human DNA (34, 35) and restriction maps of relevant segments indicating the portions used as unique sequence probes are presented in Fig. 1. N-ras: The derivation of a 900 bp Pvu II fragment which is homologous to the first exon of c-H-ras-1 has been described (36). This probe detects a single 9 kbp band in Eco RI-digested human DNA. It hybridizes weakly with homologous rodent sequences but no other human ras family members are detected under stringent hybridization conditions (Fig. 2a). c-K-ras: The isolation and detailed characterization of c-K-ras-1 and c-K-ras-2 clones will be described elsewhere (R. Gol et al. in preparation). In the present studies a 2.4 kbp Eco RI fragment of human DNA containing a 250 bp sequence homologous to the 3' end of v-K-ras was subcloned from a 20 kbp region of the human c-K-ras-2 locus (clone 482-1). This probe detected a 2.4 kbp c-K-ras-2 specific band in Eco RI digested human DNA and also a 3.2 kbp band characteristic of c-K-ras-1 (Fig. 1). A second c-K-ras clone, 482-16, was also used in some studies and represents sequences in v-K-ras about 300 bp upstream from those homologous to the c-K-ras-2 clone 482.1. c-myb: A 2.6 kbp Eco RI fragment containing 300 bp of v-myb-specific sequences was employed (37). Each DNA fragment was subcloned in either pBR332 (N-ras, myb) or pAT153 (c-K-ras). Gel purified inserts were labelled with ³²P-dCTP by nick-translation.

DNA Isolation and Filter Hybridization

DNA was isolated from each hybrid cell line (38), digested with a suitable restriction enzyme, size fractionated by (0.8%) agarose gel electrophoresis, and transferred to nitrocellulose or diazobenzoxymethyl-derivatized

Figure 1

Molecular clones of human oncogenes. Representative restriction endonuclease cleavage sites (Ba=Bam HI, B=Bgl II, E=Eco RI, H=Hind III, S=Sac I, and X=Xba I) and regions of homology (black boxes) with the corresponding viral oncogene are shown for each clone. The double-headed arrow under each map indicates the region which was subcloned and used as a hybridization probe. The transcriptional orientation of the maps is left (5') to right (3').

paper for molecular hybridization under stringent conditions (30) and then autoradiographed.

RESULTS

Chromosomal Mapping of N-ras

In one series of human/hamster hybrid cell lines, the N-ras sequence was detected in eight lines (Fig 2A). Examination of the chromosome content of these hybrids (Fig 3) indicated that the N-ras sequence could be located on either human chromosomes 1 or 5. Analysis of three additional series of somatic cell hybrids and subclones demonstrated that the presence of N-ras correlated only with the presence of human chromosome 1 (Table 1). Discrepancies were observed only in one series (b, Table 1) involving three subclones all derived from a single human/hamster somatic cell hybrid. These

Figure 2

Southern hybridization of ^{32}P -labeled human *c-onc* probes with restriction endonuclease digested, size-fractionated DNA isolated from somatic cell hybrids and controls. A. Hybridization of *Eco* RI digested DNA from hybrid series a (Table 1 and Fig 3) with *N-ras* probe (485-4) detects a 9Kb human fragment. B. Hybridization of *Eco* RI cut DNA from hybrid series c (Fig 4) with *c-K-ras-2* probe (clone 482-1) shows 2.4Kb *c-K-ras-2* and 3.2Kb *c-K-ras-1* human sequences. C. The *c-myb* probe (416-1) hybridizes with a 4.2 Kb band in *Bam* HI-digested human DNA and hybrid series c (Fig 4).

Figure 3

Distribution of specific human chromosomes in human/hamster hybrid cell lines of series a (Table 1-4). Hybrids were isolated after fusion of human fibroblasts (GM0073) containing a reciprocal X;14 translocation with *hprt*⁻ Chinese hamster fibroblasts as described (39). Individual hybrid cell lines are represented on the ordinate and H or T indicated expansion of that cell line in HAT (100 μ M hypoxanthine, 1 μ M aminopterin, and 16 μ M thymidine) or 50 μ M 6-thioguanine, respectively. Specific human chromosomes are represented on the abscissa. Solid boxes indicates the presence of a particular human chromosome as determined by isoenzyme analyses. The presence of specific cellular oncogenes is indicated by the gray dotted boxes.

three lines exhibited all markers (i.e. 6-PGD, PGM-1, and ENO-1) for the human chromosome 1p (i.e. short arm) but they did not express a chromosome 1q (i.e. long arm) marker (FH) and they lacked the human *N-ras* sequence. It is reasonable to conclude that a translocation involving human chromosome 1 has occurred in these lines and that *N-ras* is not located on the portion of this chromosome which is retained. In addition, one human/mouse hybrid subclone did not express a distal chromosome 1q marker (Pep C) and retained the *N-ras* sequence. The chromosome 1 break points in these hybrids have not been ascertained by karyotypic analysis.

Chromosomal Location of Human c-K-ras-1 and c-K-ras-2

Although c-K-ras-1 and c-K-ras-2 share considerable sequence homology, restriction fragments of each gene could be readily distinguished in digests of human DNA by using the c-K-ras-2 probe. Thus, c-K-ras-1 and c-K-ras-2 were represented, respectively, as 3.2 kbp and 2.4 kbp *Eco* RI fragments in human placenta DNA. Other members of the human *ras* family were not detected under the stringent hybridization conditions employed. The probe also detected rodent c-ras homologues in digests of rodent and hybrid cell DNAs; however, these fragments were readily resolved from the human homologues (Fig. 2B).

TABLE 1
Segregation of Neuro-ras gene with specific human chromosomes

Series	Total Hybrids	N-ras Positive	% Discordancy	
			Chromosome 1	Other Chromosomes
a	18	8	0	≥22 ^a
b	19	6	16	≥16 ^b
c	12	6	0	≥25
d	20	17	0	≥10 ^d

Assignment of the normal allele of the N-ras gene to human chromosome 1 is based on analysis of the segregation of this gene with specific human chromosomes in human-rodent somatic cell hybrids. Hybrids series a (Fig 3) and b represent lines isolated after fusing human fibroblasts containing a reciprocal X;14 chromosome translocation with hprt⁻ Chinese hamster fibroblasts as described (21, 39). Series c refers to 4 subclones of a human-mouse hybrid line and 8 independent hybrid lines isolated after fusing human fibroblasts containing a reciprocal 2;6 chromosome translocation with hamster cells (Fig 4). Series d denotes subclones of two additional hybrid cell lines. Failure to retain or lose both the N-ras gene and a particular human chromosome represents discordancy. a, exceptions were chromosomes 5 (0%) and 18 (11% discordancy). b, exception was chromosome 17 (11%); the 3 discordancies (16%) with chromosome 1 arise from a translocation of this chromosome (see text). d, exception was chromosome 19 (5% discordancy).

Analysis of two groups of hybrids (Figs 3 and 4) demonstrated that these genes were located on different human chromosomes. Examination of additional hybrid cell lines provided an unambiguous assignment of c-K-ras-2 (Table 2) and c-K-ras-1 (Table 3) to human chromosomes 12 and 6, respectively. Use of hybrids prepared from a human parental line containing a well-characterized reciprocal chromosome 2;6 translocation permits regional assignment of the c-K-ras-1 gene to chromosome 6p or the centromeric region of 6q proximal to 6q15 (Fig 4). This conclusion was confirmed by examining a series of ten subclones of a human/hamster hybrid containing only human chromosome 22 and part of chromosome 6. Giemsa banding analysis indicates that the entire chromosome 6 short arm (6p) is retained in this hybrid with the break point just below the centromere in band 6q13 (Fig. 5). All ten subclones retained the 3.2 kbp human c-K-ras-1 hybridizing band and they expressed human PGM-3 and hybridized with a human HLA probe which is a marker for 6p. These hybrid cells did not express soluble human malic enzyme (ME-1) indicating that the

Figure 4

Distribution of specific human chromosomes in human/hamster hybrid cell lines of series c (Tables 1-4). Hybrids were isolated after fusion of human fibroblasts (GM2658) containing a reciprocal 2;6 translocation with *hprt*⁻ Chinese hamster fibroblasts. Individual hybrid lines are represented on the ordinate and specific human chromosomes are depicted on the abscissa. Solid boxes indicates the presence of a particular chromosome and dotted boxes represent the presence of a *c-onc* gene. The presence of the short arm (p) and long arm (q) chromosome 2 fragments in the form of reciprocal t (2;6) translocation chromosomes was inferred by the presence of MDH-1 and ACP-1 or IDH-1, respectively. The presence of predominantly short arm and long arm portions of human chromosome 6 was inferred by the expression of human ME-1 and PGM-3 or SOD-2, respectively.

TABLE 2
Segregation of *c-K-ras-2* gene with specific human chromosomes

Series	Total Hybrids	<i>c-K-ras-2</i> Positive	% Discordancy	
			Chromosome 12	Other Chromosomes
a	18	6	0	≥11 ^a
b	19	9	0	≥16 ^b
c	12	7	0	≥25
d	30	11	0	≥27 ^d
e	15	6	0	≥33 ^e

Detection of the *c-K-ras-2* gene correlated with the presence or absence of human chromosome 12 in all hybrid cell lines. Series e represents subclones of two human-mouse hybrid lines and d indicates subclones of one additional human-mouse and 2 human-hamster hybrid lines. Series a, b, and c are described in Table 1. a, exception was chromosome 3 (0%). b, exceptions were chromosomes 15 (0%) and 10 (5% discordancy). d, exceptions were chromosomes 14 (0%), 8 (7%), and 5 (13%). e, no discordancy with Pep B (12q) marker but all discordant with LDH-B (12p) marker (see text).

TABLE 3
Segregation of c-K-ras-1 gene with specific human chromosomes

Series	Total Hybrids	c-K-ras-1 Positive	% Discordancy	
			Chromosome 6	Other Chromosomes
a	18	18	0	$\geq 28^a$
c	12	6	0	$\geq 25^c$
d	30	30	0	$\geq 43^d$
e	15	3	0	$\geq 20^e$

Detection of the c-K-ras-1 gene correlated with the presence or absence of human chromosome 6. Hybrid series are described in Tables 1 and 2. a, exceptions were chromosomes 15 (0%) and 21 (17%). c, exception was chromosome 1 (17%); one discordancy with chromosome 6q but none with 6p (see text). d, no discordancy with chromosome 6p but 10 (33%) discordancies with 6q (see text). e, exception was chromosome 4 (13%).

locus for human ME-1 is distal to 6q13.

The c-K-ras-2 gene could be regionally assigned to the long arm of human chromosome 12 (12q). Fifteen subclones (Table 2, e) derived from two WI 38 human/mouse hybrid cell lines were examined. These two parental hybrid lines were cloned from a single plate and we now believe that they arose from a single hybrid. None of these subclones express the human LDH-B chromosome 12p marker. Six subclones express the human chromosome 12q (Pep B) isoenzyme marker and exhibit the 2.4 kbp c-K-ras-2 band. Since there is a fragile site

Figure 5

The G-banded truncated human chromosome 6 from four metaphases of human/hamster hybrid cell line AV/CHT-SCL is shown (a). A G-banded normal human chromosome 6 is also presented (b). The idiogram (c) illustrates the breakpoint at 6q13 producing the truncated chromosome 6 in this hybrid cell line.

Figure 6. Metaphase preparation of a human-mouse hybrid stained by the giemsa-11 technique (40). Mouse chromosome arms are dark and centromeres light; human chromosomes exhibit the reverse pattern. Short arrows point to human chromosomes and the long arrow indicates a human-mouse translocation chromosome.

on the chromosome 12 long arm (12q13) proximal to the Pep B locus, we assume that the chromosome break has occurred at this site. Alkaline giemsa karyotypic analysis (Fig 6) of three of these subclones demonstrates a similar human/mouse translocation chromosome in each metaphase and it appears to lack a human centromere. This translocation chromosome was not detected in subclones which did not express human Pep B. Precise identification of the chromosome 12 break point awaits completion of our giemsa banding analysis.

Most of these hybrid cell lines were also analyzed with a 3 kbp *Eco* RI probe (Fig 1) representing the 5' exons of the *c-K-ras-2* gene (clone 482-16). These results (not shown) confirm the assignment of this gene to human chromosome 12.

TABLE 4
Segregation of c-myb with specific human chromosomes

Series	Total Hybrids	<u>c-myb</u> Positive	% Discordancy	
			Chromosome 6	Other Chromosomes
a	18	18	0	$\geq 28^a$
c	8	4	0	$\geq 25^c$
d	19	9	0	$\geq 32^d$
e	5	3	0	$\geq 40^e$

Detection of c-myb correlated with the retention or loss of human chromosome 6. a, exceptions were chromosomes 15 (0%) and 21 (17%). c, exceptions were chromosomes 3, 14, and 6p (13%); see text. d, except no discordancies with chromosomes 1, 3, 7, 10, 15, 19, 20, and 21; 5% discordancy with chromosomes 2 and 17; 11% discordancy with chromosome 11. e, exceptions were chromosomes 14 and 15 (0%) and 13 and 18 (20% discordancy).

Regional Localization of c-myb

The human c-myb probe (Fig 1, clone 416-1) detects a 4.2 Kb Bam H1 fragment in human DNA (Fig 2c). We examined several series of hybrids and the results (Table 4) indicate that the c-myb gene is located on chromosome 6 as previously reported (26). Concordant segregation of the c-myb and c-K-ras-1 genes was observed in all hybrids except those containing breaks or translocations involving human chromosome 6. Hybrids containing the well characterized chromosome 2;6 reciprocal translocation are informative for the regional localization of c-myb (Fig 4) and indicate that this gene is located on the long arm of chromosome 6 (6q) distal to the break point at 6q15. This interpretation is supported by analysis of the ten hybrid subclones containing 6p and the proximal portion of the long arm (6pter - 6q13). Whereas all these subclones contain the c-K-ras-1 sequence, none of them hybridize with the c-myb probe.

Nine subclones of a HeLa/mouse hybrid retained many human chromosomes including chromosome 6. These subclones all express two human chromosome 6 isoenzyme markers (ME-1 and PGM-3) but they do not exhibit a third marker (SOD-2) for this chromosome. The human c-myb gene was detected in all these subclones suggesting that it is located proximal to the SOD-2 locus at 6q21.

DISCUSSION

Members of the ras family of onc genes have been implicated as being potentially important in processes leading normal cells to become malignant. By transfection analysis, ras related oncogenes have been frequently detected as transforming genes in a variety of human tumors (14-20, 41-44). We have previously mapped c-H-ras-1, the cellular gene that gave rise to the T24/EJ bladder carcinoma oncogene, to human chromosome 11 (21). Recent studies by other groups have mapped N-ras (44), c-K-ras-2 (23,45), and c-K-ras-1 (23) to other human chromosomes. Our present studies confirm and extend these findings, providing evidence on the regional chromosomal localization of these genes as well. We have shown that the c-K-ras-2 gene is located on the long arm of human chromosome 12 (12q) and it is probably located distal to the fragile site at 12q13. Of interest is the fact that an extra chromosome 12 has been reported to occur with high frequency in peripheral blood lymphocytes from patients with chronic B-cell lymphocytic leukemia and it has been suggested that the important segment is 12q13 to 12q22 (46). The c-K-ras-2 locus has been identified as the transforming gene in a human adenocarcinoma of the colon (45) and in many different human tumor cell lines including lung, colon, pancreas and bladder carcinomas and rhabdomyosarcoma (14, 41-43). The homologous mouse sequence has also been identified as a frequent transforming gene in methylcholanthrene-induced mouse fibrosarcomas (47).

The present study indicates that the human c-K-ras-1 sequence is present on the short arm of chromosome 6 or the centromeric region (6pter-6q13). The only reported chromosome anomaly involving the short arm of chromosome 6 is a high incidence of iso-chromosome 6p in retinoblastoma (48). In contrast to c-K-ras-2, the c-K-ras-1 sequence has never been detected as a human transforming gene and it was recently reported (49) to represent a processed pseudogene containing numerous deletions, insertions, and nucleotide substitutions. It was also reported to contain translational termination codons in all reading frames thereby precluding synthesis of a functional p21 ras protein if it were activated.

An activated N-ras sequence has been identified as the transforming gene in a human neuroblastoma line (20), two fibrosarcoma lines (44), and several hematopoietic malignancies (36, 50). The localization of the N-ras gene on chromosome 1 is interesting since a terminal deletion of the short arm of this chromosome (1p31 - 1pter) was reported in 3 of 6 neuroblastomas and neuroblastoma cell lines examined by Brodeur et al. (51). Our results con-

firm that N-ras is located on chromosome 1. The absence of this gene in three hybrids containing markers for the distal portion of the short arm of chromosome 1 suggests that N-ras is located on the proximal half of this arm (i.e. below 1p22) or on the long arm proximal to the Pep C locus. However, our regional localization of N-ras is quite provisional based on the small number of informative hybrids and absence of karyotypic analysis.

Two non-random aberrations of the long arm of chromosome 6 have been reported in association with specific human neoplasms. A 6;14 chromosome translocation involving band 6q21 has been reported in ovarian cancer and a 6q21-6q25 deletion has been observed in acute lymphoblastic leukemia (52). Harper et al. (53) recently localized c-myb to the 6q22-6q24 region by in situ hybridization. Our results unambiguously localize this gene distal to 6q15 and strongly suggest that it is proximal to the SOD-2 locus (6q21). Thus, our studies and those of Harper et al. localize the c-myb gene to a narrow region in close proximity to the chromosome break point in these neoplasms.

The correlation observed between human chromosomes bearing c-onc genes and those containing non-random aberrations in specific human neoplasms raises the question as to whether a causal relationship between oncogenes and the neoplastic process in those tumors can be established. Available evidence indicates that while some c-onc genes and chromosomal aberrations map to the same chromosome bands, others do not (52). Moreover, the present limits for the mapping procedures only approaches 1000 Kb. As yet, the only cellular onc gene which has been mapped at the nucleic acid sequence level to the same region as a human chromosome translocation or deletion is c-myc, and proximity has not been shown to be invariant (2-7). The 8;14 translocation in Burkitt's lymphoma apparently results in enhanced transcriptional activity of the c-myc gene independent of detectable molecular rearrangement of this gene (6).

Since completion of this work, three papers have appeared which also localize some human ras gene family members. de Martinville et al. (54) have assigned N-ras to the proximal portion (1p3200 → cen) of the short arm of chromosome 1 based on analysis of somatic cell hybrids containing spontaneous breaks and translocations involving this chromosome. Ryan et al. (24) have reported similar regional localization (1 cen → p21) by in situ hybridization. Both our work and Ryan et al. (24) assign c-K-ras-2 to human chromosome 12 but their results suggest that it is located on the short arm. Jhanwar et al. (25) chromosomally mapped human ras genes by in

situ hybridization using Harvey and Kirsten viral onc probes under conditions of relaxed stringency. They detected ras-related sequences on chromosome 11p and both arms of chromosome 12. However, ras sequences were not detected on chromosome 6 in contrast to our results and those of O'Brien et al (23). At present, the reasons for these discrepancies are unclear but they may relate to differences in the probes used and hybridization conditions.

Ras related human oncogenes so far analyzed have all been shown to be activated as the result of point mutations in the coding regions of the genes (17-20, 44, 55, 56). Such subtle changes are not likely to be due to chromosomal aberrations as gross as translocations or deletions detectable by banding techniques. In vitro linkage of a retroviral LTR to the normal allele of at least one member of the ras family has been reported to lead to its acquisition of transforming properties (57). These findings have been interpreted to mean that increased expression of the normal ras allele can cause transformation (57). If so, tumors in which regional localization of ras oncogenes coincides with specific chromosomal aberrations would seem to warrant investigation to determine whether they are associated with a major increase in ras expression.

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