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Genetics

Chromosomal assignment of a family of human oncogenes

(somatic cell hybrids/c-*ras*)

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ABSTRACT **A family of human transforming genes, previously shown to share homology with the *ras* family of viral oncogenes, maps to three different human chromosomes. A well-characterized mouse–human hybrid cell panel, combined with Southern blotting, was used in this study. The transforming gene of the T24 bladder carcinoma cell line maps to human chromosome 11. An oncogene isolated from the lung carcinoma cell line SK-Calu-1 maps to human chromosome 12. The third *ras*-related gene, cloned from SK-N-SH, a neuroblastoma cell line, maps to human chromosome 1.**

The study of tumorigenic RNA retroviruses has led to the discovery of discrete genetic entities that are capable of transforming normal cells to a state of malignant growth (1). These DNA sequences have been termed oncogenes. Additionally, viral oncogenes are homologous to endogenous cellular genes (2, 3). It is widely speculated that the transforming genes of RNA viruses represent transduced cellular genes. Recent studies support this hypothesis (4, 5). The roles of these cellular protooncogenes in normal cell metabolism are unknown.

A second avenue of investigation, utilizing DNA-mediated gene transfer, also has led to the cloning of oncogenic sequences (6–9). However, these oncogenes are isolated from mammalian tumor cell lines, not viruses. Interestingly, many of the oncogenes isolated in this manner are homologous to viral oncogenes (10–13). These cellular oncogenes are mutated protooncogenes (14–16). It is possible that these mutations are the ultimate molecular cause of cellular transformation.

A number of oncogenes have been mapped to human or mouse chromosomes (17–22). Klein (23) predicted that chromosome alterations characteristic of certain cancers may involve rearrangements of cellular oncogenes, resulting in altered expression of the gene. Recent reports concerning chromosome translocations affecting the c-*myc* or c-*mos* genes in Burkitt lymphoma (24, 25), mouse plasmacytomas (26, 27), and chronic myeloid leukemia (28) confirm this idea. The map positions of other oncogenes may suggest additional correlations with chromosome changes seen in tumor cells.

In this study three human oncogenes, previously cloned from human tumor cell lines (7, 9, 13), have been assigned chromosome locations. The three genes are members of a family of genes related to the *ras* family of viral oncogenes (13). The transforming gene of the T24 bladder carcinoma cell line is closely homologous to the oncogene of the Harvey murine sarcoma virus, designated v-H-*ras*. The oncogene cloned from SK-Calu-1, a lung carcinoma cell line, is closely homologous to the oncogene of the Kirsten murine sarcoma virus, designated v-K-*ras*. The transforming gene of SK-N-SH, a neuroblastoma cell line, is weakly homologous to both viral genes and encodes a highly related protein (unpublished results). By the Southern blot technique (29), we used cloned probes of these oncogenes to screen a mouse–human hybrid DNA panel (30) and determined that (*i*) the bladder carcinoma oncogene maps to human chromosome 11, thus confirming two earlier reports (31, 32); (*ii*) the lung carcinoma oncogene maps to human chromosome 12, confirming an earlier report (33); and (*iii*) the transforming gene of the neuroblastoma line maps to human chromosome 1.

MATERIALS AND METHODS

Cell Lines. Mouse–human hybrid cells were cultivated without antibiotics in α-minimal essential medium containing 10% (vol/vol) fetal bovine serum in roller bottles. Cytogenetic and isozyme samples were taken when DNA was isolated. Monolayers were washed twice with saline and once with 50 mM Tris·HCl, pH 8/10 mM NaCl/10 mM EDTA at 4°C. This Tris/NaCl/EDTA buffer supplemented with 0.5% $NaDodSO_4$ (Bethesda Research Laboratories) and proteinase K (100 μg/ml) was added, followed by overnight incubation at 37°C on a roller mill. DNA was extracted twice with redistilled phenol, twice with chloroform/isoamyl alcohol, 24:1 (vol/vol), concentrated in dialysis bags against polyethylene glycol 8000 (Sigma), and exhaustively dialyzed against 10 mM Tris·HCl, pH 8/1 mM EDTA.

Hybrid cell parents were mouse A9–human GM144 (series AHA), mouse A9–human GM589 (series BDA), mouse A9–human 126 (series 41PT), and mouse A9–human WI38 (series WA). Subclones of hybrids were isolated by dilution plating. Genetic analysis of hybrids included G-banding of 25–50 cells for chromosome constitution and isozyme analysis. The chromosomes and their isozyme markers as determined by starch gel or cellulose acetate electrophoresis and histochemical staining, included: chromosome 1, enolase-1 (EC 4.2.1.11), phosphoglucomutase-1 (EC 2.7.5.1), and peptidase-C (EC 3.4.11.—); chromosome 2, malate dehydrogenase-1 (EC 1.1.1.37) and isocitrate dehydrogenase-1 (EC 1.1.1.42); chromosome 3, acylase-1 (EC 3.5.1.14); chromosome 4, peptidase-S (EC 3.4.11.—) and phosphoglucomutase-2 (EC 2.7.5.1); chromosome 5, hexosaminidase-B (EC 3.2.1.30); chromosome 6, glyoxylase-1 (EC 4.4.1.5) and malic enzyme-1 (EC 1.1.1.40); chromosome 7, β-glucuronidase (EC 3.2.1.31) and uridine phosphorylase (EC 2.4.2.3); chromosome 8, glutathione reductase (EC 1.6.4.2); chromosome 9, adenylate kinase-1 (EC 2.7.4.3) and aconitases (EC 4.2.1.3); chromosome 10, adenosine kinase (EC 2.7.1.20) and glutamate-oxaloacetate transaminase (EC 2.6.1.1); chromosome 11, lactate dehydrogenase-A (EC 1.1.1.27); chromosome 12, triosephosphate isomerase (EC 5.3.1.1), peptidase-B (EC 3.4.11.—), and lactate dehydrogenase-B (EC 1.1.1.27); chromosome 13, esterase-10 (EC 3.1.1.1); chromosome 14, nucleoside phosphorylase (EC 2.4.2.1); chromosome 15, mannosephosphate isomerase (EC 5.3.1.8), pyruvate kinase-3 (EC 2.7.1.40), and hexosaminidase-A (EC 3.2.1.30); chromosome

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Abbreviation: kb, kilobase pairs.

16, adenosine phosphoribosyltransferase (EC 2.4.2.7); chromosome 17, galactokinase (EC 2.7.1.6); chromosome 18, peptidase-A (EC 3.4.11.—); chromosome 19, glucosephosphate isomerase (EC 5.3.1.9); chromosome 20, adenosine deaminase (EC 3.5.4.4); chromosome 21, superoxide dismutase-1 (EC 1.15.1.1); chromosome 22, aconitase-m (EC 4.2.1.3); and the X chromosome, phosphoglycerate kinase (EC 2.7.2.3), hypoxanthine/guanine phosphoribosyltransferase (EC 2.4.2.8), and glucose-6-phosphate dehydrogenase (EC 1.1.1.49).

Molecular Probes. pT24 is pBR322 with a 6.2-kilobase-pair (kb) *Bam*HI insert, entirely of human origin, which includes the transforming gene of the T24 bladder carcinoma line (7). pLRHL4 is pBR322 with a 1.2-kb *Hin*dIII/*Eco*RI insert homologous to the transforming gene of the SK-Calu-1 lung carcinoma line (13). pNP-5 contains a 1.8-kb *Xba* I/*Eco*RI fragment, homologous to the transforming gene of the SK-N-SH neuroblastoma cell line, cloned in pchtk-2 (a pBR322 derivative containing the chicken thymidine kinase gene) (9).

Enzymes. *Bam*HI, *Eco*RI, and *Hin*dIII restriction endonucleases were purchased from New England BioLabs and Bethesda Research Laboratories and were used according to the suppliers' instructions. Nick translations were carried out by using a kit purchased from Amersham.

Southern Blotting and DNA Hybridization. DNA samples (20–30 μg) were digested with restriction endonuclease and electrophoresed in 1% agarose gels prior to transfer by the Southern technique (29) to nitrocellulose (Schleicher & Schuell) or APT (aminophenyl thioether) paper (Schleicher & Schuell). Hybridization either was done at 65–70°C for 12 hr in buffer containing 6× NaCl/Cit (1× NaCl/Cit = 0.15 M NaCl/0.015 M Na citrate, pH 7), 2× Denhardt's solution (1× = 0.02% polyvinylpyrrolidone/0.2% Ficoll/0.2% bovine serum albumin), 20 mM sodium phosphate, 10% dextran sulfate (Pharmacia), and 100 μg of denatured, sonicated salmon sperm DNA per ml or was done at 42°C for 12 hr in buffer containing 5× NaCl/Cit, 50% formamide (Macalaster Bicknell), 2× Denhardt's solution, 10% dextran sulfate, and 100 μg of denatured, sonicated salmon sperm DNA per ml, in both cases with 10 ng of denatured, nick-translated probe added per ml. Final wash in both cases was at 65°C with buffer containing 0.4× NaCl/Cit, 4 mM sodium phosphate, 0.01% sodium pyrophosphate, and 0.01% $NaDodSO_4$ (Bio-Rad). Hybridized probe was detected by autoradiography.

RESULTS

Transforming Gene of the T24 Bladder Carcinoma Cell Line Maps to Chromosome 11. Fig. 1 shows pT24 blotted against seven *Bam*HI-cut hybrid cell DNAs. A single band of human DNA of approximately 7.5 kb was seen in the HeLa cell line control track. Three hybrid DNAs, 10a3, 16e, and 3d2, also showed the 7.5-kb human DNA band. A cross-hybridizing mouse DNA band of 3.6 kb was seen in every hybrid DNA. This initial screen suggested that pT24 mapped to either chromosome 11 or 12. An assignment to chromosome 11 was made when hybridization was seen with the hybrid cell line Fry-1 (data not shown). This line has been obtained by selecting with the fluorescence-activated cell sorter for the presence of 4F2, a human cell-surface antigen that maps to chromosome 11 (34). Fry-1 DNA is characterized as having chromosome 11 as its only human chromosome. A restriction-fragment-length polymorphism was seen between Fry-1 (human parent GM 126) and HeLa DNAs (data not shown). The polymorphic nature of this locus has been noted (7). Table 1 summarizes the hybrid panel data for pT24.

Transforming Gene of the SK-Calu-1 Lung Carcinoma Cell Line Maps to Chromosome 12. Hybridization of pLRHL4 to a total of 22 hybrid DNAs (see Table 1) localized the transforming gene from the lung carcinoma cell line to chromosome 12. No cross-hybridization was seen with mouse DNA under stringent wash conditions. No polymorphisms were detected in human DNAs. To confirm this assignment, a chromosome 12 positive control was hybridized to the hybrid panel. D12S1 is a unique human restriction fragment previously mapped to chromosome 12 (35). The hybridization pattern seen with D12S1 matched that seen with pLRHL4 except for two hybrid DNAs, BDA-17b17 and 41PT2A (data not shown). Both lines are positive for the presence of pLRHL4 and negative for D12S1. Karyotypic analysis found no evidence for chromosome 12 in either line. Three chromosome 12 isozymes were scored, with negative results for peptidase B, a long-arm marker, and positive results for lactate dehydrogenase B and triosephosphate isomerase, short-arm markers, in both lines. It would appear that, although neither hybrid line has retained an intact chromosome 12, both have retained a segment of the short arm. Taken together, the results with D12S1 confirm the assignment of pLRHL4 to chromosome 12 and strongly suggest that this gene resides on the short arm.

Transforming Gene of the SK-N-SH Neuroblastoma Cell Line Maps to Chromosome 1. Fig. 2 shows pNP-5 hybridized to nine *Hin*dIII-cut hybrid cell DNAs. A single band of human DNA of approximately 10 kb is seen in the HeLa control track and in three of the nine hybrid DNAs. Hybridization to a total of 15 hybrid DNAs suggested that the neuroblastoma gene resided on either chromosome 1 or chromosome 5. To distinguish

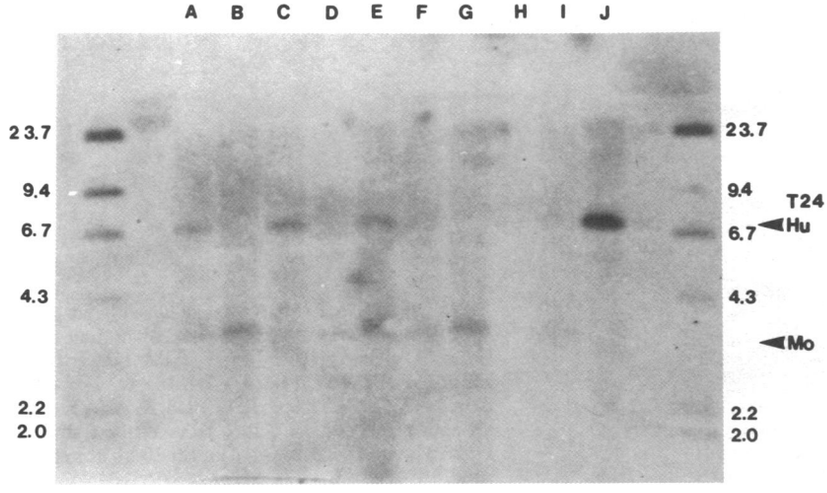

FIG. 1. pT24 (hu-H-*ras*-1) hybridized to seven *Bam*HI-cut hybrid DNAs. Tracks: A, BDA 10a3; B, BDA 17b17; C, AHA 16e; D, WAVR4d A19 F_3-2; E, AHA 3d2; F, 41PT2A; G, BDA 14b25; H, blank; I, mouse control, A9; J, human control, HeLa. Sizes are shown in kb. BDA 10a3, AHA 16e, and AHA 3d2 were scored as positive for the 7.5-kb human DNA band (Hu). A mouse DNA cross-hybridizing band of 3.6 kb is also seen (Mo).

Table 1. Correlation of blot hybridization and chromosome status in hybrid DNAs

Probes	Blot hybridization	Presence of chromosome	Chromosome content of hybrid DNAs, no. of clones																						
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X
pT24																									
(hu-H-*ras*-1)	+	+	0	3	4	1	0	1	0	2	0	3	5	3	3	1	1	2	0	2	4	3	2	1	4
	−	−	3	3	2	2	4	4	4	4	2	4	5	5	4	2	3	3	5	3	2	4	2	2	1
	−	+	2	2	3	2	1	0	1	1	2	1	0	0	1	1	2	1	0	2	3	1	2	3	4
	+	−	4	2	1	4	5	3	3	1	4	1	0	2	2	3	4	3	5	3	1	2	3	3	1
		Discordant	6	4	4	6	6	3	4	2	6	2	0	2	3	4	6	4	5	5	4	3	5	6	5
pLHRL4																									
(hu-K-*ras*-2)	+	+	1	5	8	4	2	3	0	2	0	5	5	8	2	4	2	2	2	4	6	6	6	3	9
	−	−	4	6	2	4	5	5	5	5	7	7	5	6	3	6	5	5	5	6	5	5	3	3	3
	−	+	2	1	2	2	1	2	1	0	0	0	2	0	3	0	1	1	1	1	2	2	3	3	4
	+	−	7	2	0	4	6	6	8	5	7	3	4	0	4	3	7	3	3	6	4	4	2	0	0
		Discordant	9	3	2	6	7	8	9	5	7	3	6	0	7	3	8	4	4	7	6	6	5	3	4
pNP-5																									
(hu-N-*ras*-1)	+	+	7	1	4	4	4	1	1	0	0	0	0	0	1	0	2	1	2	2	2	3	3	3	4
	−	−	11	5	2	5	11	5	8	3	8	5	4	4	5	6	7	7	6	7	6	6	4	2	2
	−	+	0	5	6	2	1	4	0	2	1	5	5	5	3	3	1	2	1	3	4	4	4	3	8
	+	−	0	1	0	0	3	3	2	4	2	4	4	1	2	1	2	2	2	2	1	1	0	1	0
		Discordant	0	6	6	2	4	7	2	6	3	9	9	6	5	4	3	3	3	5	5	5	4	4	8

Hybrid lines with inconclusive karyotype and isozyme data for a particular chromosome are not included in this table.

between these two possibilities, a series of chromosome 5^+/chromosome 5^- hybrids were created by subjecting chromosome 5^+ hybrid cells to selection with diphtheria toxin. Diphtheria toxin sensitivity maps to human chromosome 5 (36); hence, surviving cells should be chromosome 5^-. This can be verified by isozyme and karyotype analysis. Three pairs of chromosome 5^+/chromosome 5^- hybrid cell DNAs obtained in this manner were hybridized to pNP-5. The resulting band pattern (Fig. 3) did not correlate with chromosome 5. This pattern did correlate with the presence or absence of human chromosome 1. Therefore, we assigned the neuroblastoma gene to chromosome 1. Table 1 summarizes the hybrid panel data for pNP-5.

DISCUSSION

We have shown that three members of a *ras*-related family of human oncogenes map to three different chromosomes. This family of genes may be quite large: v-H-*ras* and v-K-*ras* each have two human homologs (37). The transforming gene of the T24 bladder carcinoma cell line appears to be the human homolog designated hu-H-*ras*-1 (13). The assignment of this gene to chromosome 11 confirms the work of two other laboratories (31, 32). De Martinville *et al.* (32) have localized the gene to the short arm of chromosome 11 through the use of hybrids containing translocated human chromosomes. It has been suggested, based on this localization, that this gene plays a role in the Wilm tumor/aniridia syndrome (38). Studies at the molecular level should reveal any correlation between these diseases and structural changes in this gene. The transforming gene of the SK-Calu-1 lung carcinoma cell line corresponds to the human homolog designated hu-K-*ras*-2 (13). Cytologically detectable changes in chromosome 12 have only been seen infrequently in human malignancies (39). It is interesting that hu-H-*ras*-1 and hu-K-*ras*-2 map to the short arms of chromosomes 11 and 12. These chromosomes may be related by a duplication event—for example, the gene for lactate dehydrogenase A, *LDH-A*, resides on the short arm of chromosome 11, and *LDH-B* is found on the short arm of chromosome 12 (30). The transforming gene of the SK-N-SH cell line, termed hu-N-*ras*-1, represents a third branch of this family (9, 13). There appear to be additional human genes closely related to hu-N-*ras*-1 (13). It may be relevant that chromosome 1 anomalies have been noted in several human neuroblastomas (40, 41). In addition, chromosome 1 changes occur frequently in various hematologic neoplasias (42). It remains to be determined if the additional genes of this family are dispersed throughout the genome or if members of a particular branch are clustered together.

The three *ras*-related human transforming genes mapped here have been isolated frequently from tumor lines and solid tu-

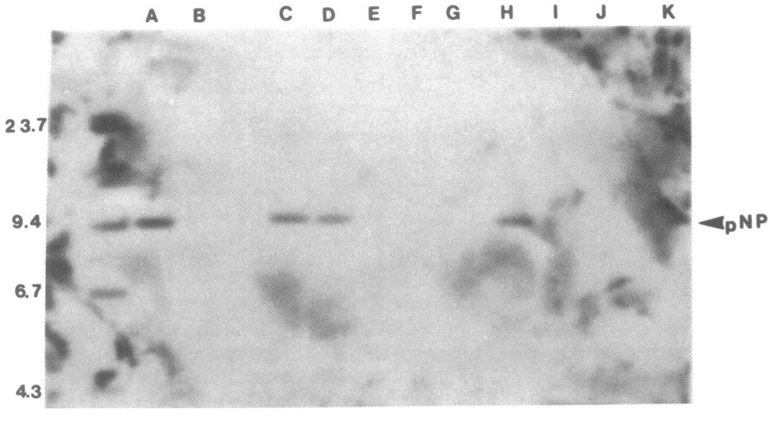

FIG. 2. pNP-5 (hu-N-*ras*-1) hybridized to nine *Hin*dIII-cut hybrid DNAs. Tracks: A, human control, HeLa; B, mouse control, A9; C, BDA 17b17; D, BDA 17b17-6; E, BDA 17b17-3; F, AHA 16e; G, AHA 3d2; H, BDA 14b25; I, 41PT2A; J, BDA 10a3; K, AHA 11a. Sizes are shown in kb. BDA 17b17, BDA 17b17-6, and BDA 14b25 were scored as positive for the 10-kb human DNA band.

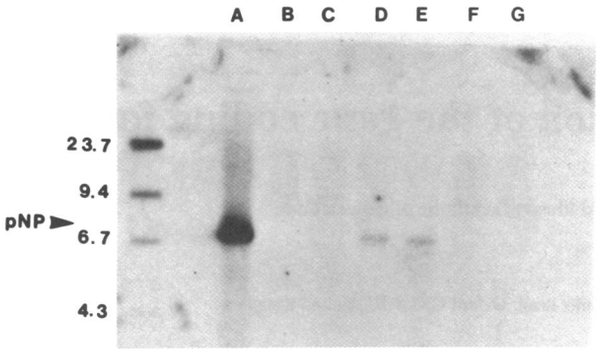

FIG. 3. pNP-5 (hu-N-*ras*-1) hybridized to three pairs of diphtheria toxin-selected chromosome 5^+/chromosome 5^- *Eco*RI-cut DNAs. Tracks: A, human control, HeLa; B, BDA 10a3; C, BDA 10a3 DT; D, BDA 17b17-1; E, BDA 17b17-1 DT; F, AHA 16e; G, AHA 16e DT. Sizes are shown in kb. BDA 10a3, BDA 17b17-1, and AHA 16e are positive for chromosome 5. BDA 10a3 DT, BDA 17b17-1 DT, and AHA 16e DT are negative for chromosome 5. BDA 17b17-1 and BDA 17b17-1 DT were scored as positive for the 7.0-kb human DNA band.

mors (43). This may be due to an inherent bias in the NIH 3T3 assay or may reflect an important role of this family of genes in human carcinogenesis.

Note Added in Proof. The hu-N-*ras*-1 gene locus has subsequently been localized by M. Rabin in our laboratory to 1 cen → p21 by hybridization *in situ* of an N-*ras* specific probe to human metaphase chromosomes.

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