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Genetics

Chromosomal assignment of the human homologues of feline sarcoma virus and avian myeloblastosis virus *onc* genes

(somatic cell hybrids/Southern blotting technique/human cancer)

RICCARDO DALLA-FAVERA*, GENOVEFFA FRANCHINI*, STEFANO MARTINOTTI*, FLOSSIE WONG-STAAL*, ROBERT C. GALLO*, AND CARLO M. CROCE†

*Laboratory of Tumor Cell Biology, National Cancer Institute, Bethesda, Maryland 20205; and †The Wistar Institute of Anatomy and Biology, 36th Street at Spruce, Philadelphia, Pennsylvania 19104

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ABSTRACT **Retroviral transforming genes, v-*onc* genes, are derived from normal cellular sequences that are called cellular *onc* (c-*onc*) genes. DNA from mouse–human somatic cell hybrids that have selectively lost human chromosomes was used in Southern blots to map the chromosomal location of two human *onc* genes. Cloned human homologues of retroviral *onc* genes were used as probes. Because the human c-*fes* gene, which is homologous to feline sarcoma virus, segregates concordantly with human chromosome 15, and the human c-*myb* gene, which is homologous to avian myeloblastosis virus *onc* genes, segregates concordantly with human chromosome 6, we have assigned the c-*fes* and the c-*myb* genes to human chromosomes 15 and 6, respectively. Nonrandom chromosomal defects involving these human chromosomes have been observed in neoplasms. These studies should be valuable in determining whether specific rearrangements involving these chromosomes result in the abnormal expression of these *onc* genes in human malignancies.**

Substantial evidence indicates that retroviral transforming genes (v-*onc* genes) originated by recombination between a parent nontransforming virus and normal cellular sequences (c-*onc* genes) (1). Cellular *onc* genes are highly conserved during evolution (1), consistent with the notion that they may code for basic functions in cell growth or tissue differentiation. Several examples suggest that in some cases neoplastic transformation may be due to an increased level of *onc* gene expression. *In vitro* experiments performed with cloned mouse c-*mos*, the cellular homologue of Moloney sarcoma virus (2), showed that this gene acquires transforming activity when its expression is enhanced by the linkage to promoter sequences from the murine leukemia virus genome (2). *In vivo*, B-cell lymphomas in chickens are characterized by abnormal expression of another cellular *onc* gene, c-*myc*, homologous to the transforming gene of avian myelocytomatosis virus (MC29) (3–6). In this model of direct retroviral transformation the cellular *onc* gene is switched on because it is activated by a retrovirus promoter (3–6). Because certain human and animal tumors display specific chromosomal rearrangements suggesting a close relationship between a specific genomic alteration and neoplastic transformation (7, 8), an alternative mechanism to explain the abnormal expression of an *onc* gene could be its chromosomal translocation to a region of the cellular genome that is transcriptionally active, leading to abnormal levels of its expression. We have initiated a series of investigations designed to establish the chromosomal localization of several known c-*onc* genes on normal human chromosomes as a first step toward the analysis of their possible rearrangement in tumor cells. In this study we report the chromosomal assignment of two human cellular *onc* genes, homologous to feline sarcoma virus and avian myeloblastosis virus transforming genes, respectively.

Feline sarcoma viruses [Snyder–Theilen (ST), Gardner–Arnstein (GA), and Sarma-McDonough (SM) strains] are a family of acutely transforming retroviruses isolated from naturally occurring sarcomas of cats (9–11). ST and GA feline sarcoma virus strains owe their *in vivo* and *in vitro* transforming potential to the same *onc* gene, called v-*fes*, which has been acquired from cat cellular sequences (c-*fes*) (12). c-*fes* sequences have been detected in the human genome (13), and recently the c-*fes* locus has been isolated in a recombinant phage and characterized (14, 15). Avian myeloblastosis virus is also an acutely transforming virus (16) whose *onc* gene, v-*myb*, confers upon the virus the ability to transform *in vitro* a specific class of hematopoietic cells and to induce myeloblastic leukemia in chickens (16). The human cellular homologue, c-*myb*, of the avian myeloblastosis virus *onc* gene has been shown to be transcribed into RNA in human immature hematopoietic tissues (17), and different recombinant phages containing c-*myb* sequences have been isolated from a human DNA library (unpublished data).

Using c-*fes* and c-*myb* probes, we have examined human–mouse cell hybrid DNAs with different numbers and combinations of human chromosomes on Southern blots (18) for the presence of human *onc* sequences. The data established that the c-*fes* and c-*myb* loci are on human chromosomes 15 and 6, respectively.

MATERIALS AND METHODS

Somatic Cell Hybrids. Panels of mouse–human somatic cell hybrids between mouse fibroblasts or myeloma cells and human fibroblasts or B cells were included in this study. In the panels we included hybrids that contained single human chromosomes (19) or only pairs of human chromosomes (20). One of these hybrid clones (clone 13) contained only a human chromosome 11 attached to a small segment of the human X chromosome (21). This hybrid derived from the fusion of a mouse cell line deficient in the enzyme hypoxanthine phosphoribosyltransferase (HPRT) and skin fibroblasts derived from a patient with gonadal dysgenesis carrying a t(X;11)(25-26;23) chromosomal translocation (21). An additional hybrid clone, Nu-9, that contained only human chromosomes 6 and 7 (20) was subcloned, and two subclones (Nu-91 and Nu-92) that have lost human chromosome 6 and retained human chromosome 7 were included in this study.

Isozyme Analysis. Hybrid cells were studied for the expression of isozyme markers assigned to each of the different human

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Abbreviation: kb, kilobase(s).

chromosomes by starch gel or cellulose acetate gel electrophoresis: 1, enolase 1 (EC 4.2.1.11); 2, isocitrate dehydrogenase (EC 1.1.1.42); 3, β-galactosidase (EC 3.2.1.23); 4, phosphoglucomutase 2 (EC 2.7.5.1); 5, hexosaminidase B (EC 3.2.1.30); 6, glyoxalase 1 (EC 4.4.1.5) and phosphoglucomutase 3 (EC 2.7.5.1); 7, β-glucuronidase (EC 3.2.1.31); 8, glutathione reductase (EC 1.6.4.2); 9, aconitase (EC 4.2.1.3); 10, glutamate–oxaloacetate transaminase (EC 2.6.1.1); 11, lactate dehydrogenase A (EC 1.1.1.27); 12, lactate dehydrogenase B (EC 1.1.1.27); 13, esterase D (EC 3.1.1.1); 14, nucleoside phosphorylase (EC 2.4.2.1); 15, mannosephosphate isomerase (EC 5.3.1.8); 16, adenine phosphoribosyltransferase (EC 2.4.2.7); 17, galactokinase (EC 2.7.1.6); 18, peptidase A (EC 3.4.11.-); 19, glucosephosphate isomerase (EC 5.3.1.9); 20, adenosine deaminase (EC 3.5.4.4); 21, superoxide dismutase 1 (EC 1.15.1.1); 22, arylsulfatase (EC 3.1.6.1); X chromosome, glucose-6-phosphate dehydrogenase (EC 1.1.1.49).

DNA Extraction and Southern Blot Analysis. DNA from various mouse, human, and hybrid cell lines was prepared by cell lysis, proteinase K digestion, extraction with phenol, and precipitation with ethanol as described (22). Thirty micrograms of DNA was digested with 60 units of the appropriate restriction endonuclease in standard conditions recommended by the supplier (New England BioLabs). Fragments were separated by electrophoresis on a 0.8% agarose gel. DNA was denatured and transferred to nitrocellulose as described by Southern (18). Hybridization and autoradiography were performed according to Wahl *et al.* (23), except that filter washing was performed in 0.45 M NaCl/0.045 M sodium citrate/0.5% sodium dodecyl sulfate, pH 7, at 60°C for 2 hr.

Probes for Hybridization. *Human c-*myb *probe.* A recombinant phage containing sequences homologous to avian myeloblastosis virus *onc* gene (v-*myb*) was isolated (unpublished results) from a human recombinant DNA library (24), using cloned v-*myb* sequences as a probe (a gift from M. Baluda). *Eco*RI digestion of phage DNA generated several bands in the human DNA insert but the viral probe (17) hybridized only to a 2.0-kilobase (kb) fragment. The 2.0-kb fragment containing c-*myb* sequences was isolated by preparative gel electrophoresis (25), ligated to *Eco*RI-digested pBR322 DNA (26), and transfected into *Escherichia coli* K802. Colonies containing the recombinant plasmid were detected by replica plating and colony hybridization methods (27).

*Human c-*fes *probe.* A recombinant phage containing human c-*fes* sequences (15) contains a 3.9-kb *Bam*HI fragment homologous to the 5′ fraction of the v-*fes* gene (15, 28). This fragment was inserted into pBR322 plasmid as described above.

c-*myb* (clone pF8) and c-*fes* (clone pN26) recombinant plasmids were used as probes after labeling with [^{32}P]dCTP and [^{32}P]dATP by nick-translation (29).

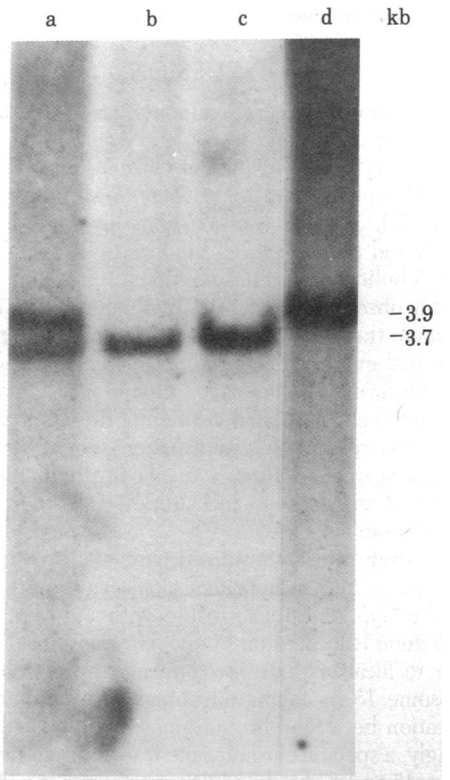

FIG. 1. Hybridization of human c-*fes* probe to mouse, human, and mouse–human hybrid cell line DNAs. Lane a, hybrid DNA (57-77 F7 DC7); lane b, negative hybrid DNA; lane c, mouse DNA; lane d, human DNA. See text for description of hybrid cell lines.

RESULTS

c-*fes* Mapping. Southern blot experiments using the c-*fes* recombinant plasmid (clone pN26) as a probe showed a single 3.9-kb band in *Bam*HI-digested human DNA (Fig. 1). Several human cell lines, including the parental cell lines used for the cellular hybrids, displayed the same band (data not shown). Under nonstringent conditions of hybridization (see *Materials and Methods*) the probe hybridized to a single 3.7-kb DNA fragment in different mouse parental cell lines analyzed, confirming the evolutionary conservation of c-*fes* sequences. Thus by *Bam*HI digestion it was possible to distinguish the human c-*fes* fragment from the murine homologue, allowing us to screen DNAs from human–mouse hybrid cells. DNAs from all cell hybrids contained the 3.7-kb mouse fragment, whereas only some had the 3.9-kb human fragment (see Fig. 1 for repre-

Table 1. Segregation of the human c-*fes* gene in mouse–human hybrids

Human chromosome	+/+	+/−	−/+	−/−
1	1	6	2	19
2	0	7	4	17
3	3	4	2	19
4	5	5	7	18
5	2	6	4	16
6	4	3	4	17
7	1	6	9	12
8	1	6	1	20
9	1	6	3	18
10	2	5	1	20
11	8	3	2	22
12	2	8	13	12
13	3	4	2	25
14	6	1	13	8
15	10	0	0	25
16	1	6	1	20
17	4	3	4	17
18	2	5	2	19
19	1	6	4	17
20	1	6	3	18
21	1	6	1	20
22	1	6	6	15
X	3	4	2	19

Numbers in columns indicate the number of hybrid cell lines tested that contain both the c-*fes* gene and the numbered chromosome (+/+), the c-*fes* gene but not the numbered chromosome (+/−), the numbered chromosome but not the c-*fes* gene (−/+), or neither the numbered chromosome nor the c-*fes* gene (−/−).

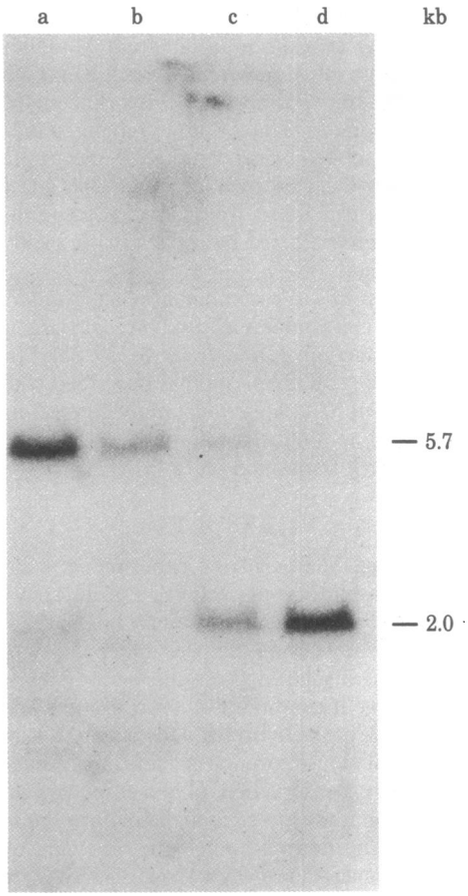

FIG. 2. Hybridization of human c-*myb* probe to mouse, human, and mouse–human cell line DNAs. Lane a, negative hybrid DNA; lane b, mouse DNA; lane c, positive hybrid DNA (clone Nu-9); lane d, human DNA. See text for description of hybrid cell lines.

sentative data). The absence of the human fragment indicated the absence of the human chromosome on which the c-*fes* gene is located.

Positive correlation between the presence of the human c-*fes* fragment and the presence of human chromosome 15 is shown in Table 1. Negative correlation was observed with all other human chromosomes. DNA from clone 57-77 F7 DC7, a hybrid that carried only human chromosomes 5, 13, 14, 15, and X and contained the human c-*fes* gene, is shown in lane a of Fig. 1. These results indicate that the c-*fes* gene is located on human chromosome 15.

c-*myb* Mapping. A 2.0-kb fragment was visible when probe pF8 was hybridized to *Eco*RI digests of human DNA (Fig. 2, lane d). No polymorphism of this fragment was detected in different DNA samples tested (data not shown). This fragment was clearly distinguishable from the 5.7-kb *Eco*RI detected by the same probe in different mouse parental cell lines (see Fig. 2, lane b for a representative experiment). Because it is possible to distinguish the mouse and the human c-*myb*, we screened a large number of mouse–human cell hybrids for the presence of the human sequences. These results (Table 2) indicate that the presence of the human c-*myb* gene segregates concordantly with the presence of human chromosome 6 and no other human chromosome in the hybrid clones. One of the hybrids we have studied, Nu-9, retained only human chromosomes 6 and 7 (20) and is positive for the human c-*myb* gene. Two subclones derived from this hybrid have concordantly lost human chromosome 6 and the human c-*myb* gene (data not shown). Therefore we conclude that human chromosome 6 carries the human c-*myb* gene homologue.

Table 2. Segregation of human c-*myb* gene in mouse–human hybrids

Human chromosome	+/+	+/−	−/+	−/−
1	0	8	1	18
2	0	8	3	16
3	2	6	3	16
4	1	7	2	17
5	0	8	2	17
6	8	0	0	19
7	1	7	7	12
8	0	8	5	14
9	1	7	1	18
10	0	8	1	18
11	4	4	3	16
12	3	5	3	16
13	1	7	2	17
14	6	2	9	10
15	2	6	2	17
16	0	8	2	17
17	1	7	7	12
18	1	7	4	15
19	1	7	5	14
20	3	5	2	17
21	1	7	1	18
22	3	5	3	16
X	2	6	3	16

Numbers in columns indicate the number of hybrid cell lines tested that contain both the c-*myb* gene and the numbered chromosome (+/+), the c-*myb* gene but not the numbered chromosome (+/−), the numbered chromosome but not the c-*myb* gene (−/+), or neither the numbered chromosome nor the c-*myb* gene (−/−).

DISCUSSION

Specific chromosome rearrangements have been observed in several human neoplasms (30, 31). For example, a translocation of the region q11–qter of human chromosome 22 to chromosome 9 has been observed in most cases of chronic myelogenous leukemias (30, 31), and translocations involving human chromosome 8 and the human chromosomes carrying the human immunoglobulin genes (chromosomes 2, 14, and 22) (32–35) have been observed in Burkitt lymphoma (35–37). Because chromosome translocations or rearrangements might result in the abnormal expression of cellular *onc* genes, leading to malignant transformation, we have initiated a systematic study to determine the chromosomal location of these *onc* genes in humans. In this respect this information should be valuable in directing a search for tumors displaying both high levels of expression of an *onc* gene and alterations in the chromosome where the gene is located.

In this study we have utilized panels of previously characterized mouse–human hybrids to look for the presence of the c-*fes* and c-*myb* genes. The results with a c-*fes* probe indicate that this gene is located on human chromosome 15. It may be possible to localize the c-*fes* gene more precisely on human chromosome 15 by taking advantage of hybrids containing a translocation between chromosomes 15 and 17 (38). Very interestingly, a specific chromosome translocation between chromosomes 15 and 17 has been discovered in patients with acute promyelocytic leukemia (39). Study of this chromosomal rearrangement indicates that it is a reciprocal translocation (15; 17)(q22;q21) (40). It would be of considerable interest to determine whether the c-*fes* gene is expressed in any of these

malignancies when rearrangement involving chromosome 15 is present.

We have also assigned the human c-*myb* gene to human chromosome 6. This gene has been shown to be specifically expressed in hematopoietic tissues in humans (17). High levels of c-*myb* RNA were detected in two T-cell lymphoma cell lines, CEM and MOLT-4 (41, 42). A marker chromosome (M_1) originating from chromosome 6 with a deletion of the distal two-fifths of the long arm has been described in the MOLT-4 cell line (43). Similar deletions of the long arm of chromosome 6 have been described in several cases of T-cell lymphomas and in acute lymphocytic leukemia (44). Recently, specific cytogenetic changes involving chromosomes 6 and 14, t(6;14)(q21;q24), have been described in papillary serous adenocarcinoma of the ovary (45). It should be informative to analyze the chromosomal and molecular arrangement of the c-*myb* locus in these malignant cells.

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