period of 45 to 51 minutes. Regional activity was measured for each sequential scan, corrected for ^{11}C decay, and plotted against time. The values obtained were not corrected for partial volume effects. With the camera system used, the recovery coefficient for the putamen was on the order of 0.90 (*17*). The putamen was chosen for quantitative measurements since it has the highest density of D2 dopamine receptors and the largest extension of the basal ganglia (*19*).
13. M. Kuhar, C. K. Murrin, A. T. Malouf, N. Klemm, *Life Sci.* **22**, 203 (1978).
14. M.-P. Martres *et al.*, *Science* **228**, 752 (1985).
15. C. B. Pert, M. J. Kuhar, S. H. Snyder, *Proc. Natl. Acad. Sci. U.S.A.* **73**, 3729 (1976); N. Klemm, L. C. Murrin, M. J. Kuhar, *Brain Res.* **169**, 1 (1979); C. A. Altar, S. O'Neil, R. J. Walter, Jr., J. F. Marshall, *Science* **228**, 597 (1985).
16. C. Köhler, H. Hall, S. O. Ögren, L. Gawell, *Biochem. Pharmacol.* **34**, 2251 (1985).
17. C. v. Bahr, A. Wahlén, L. Farde, G. Sedvall, in preparation.
18. J.-E. Litton *et al.*, *J. Comput. Assist. Tomogr.* **8**, 74 (1984).
19. J. Hyttel and A. V. Christensen, *J. Neural Transm. Suppl.* **18**, 157 (1983).
20. P. Seeman *et al.*, *Science* **225**, 728 (1984).
21. H. Hall and L. Farde, unpublished data.
22. The skillful technical assistance of Å. Franzén, J. Heissinger, and A. S. Ullrich is gratefully acknowledged. We also thank P. Greengard and R. Huganir for valuable comments. Supported by Swedish Medical Research Council grant 21X-03560 and the Bank of Sweden Tercentenary Fund.

17 May 1985; accepted 27 September 1985

Alterations of *myc*, *myb*, and *ras*^{Ha} Proto-oncogenes in Cancers Are Frequent and Show Clinical Correlation

JUN YOKOTA, YASUKO TSUNETSUGU-YOKOTA, HECTOR BATTIFORA, CAROL LE FEVRE, MARTIN J. CLINE

Alterations of c-*myc*, c-*ras*^{Ha}, or c-*myb* oncogenes were found in more than one-third of human solid tumors. Amplification of c-*myc* occurred in advanced, widespread tumors or in aggressive primary tumors. Apparent allelic deletions of c-*ras*^{Ha} and c-*myb* can be correlated with progression and metastasis of carcinomas and sarcomas.

PROTO-ONCOGENES MAY BE ACTIVATED and contribute to neoplastic transformation of cells through point mutations, translocations, deletions, gene amplification, or other genetic mechanisms. Mutations have been found most often in proto-oncogenes of the *ras* family, assayed by the NIH-3T3 transformation system (*1*). Chromosomal translocations involving cellular oncogenes occur in chronic myelocytic leukemias and Burkitt's lymphomas (*2*). Deletion of one allele of c-*ras*^{Ha} has been found in some Wilm's tumors (*3*) and other sporadic tumors (*4*). Amplification of several cellular oncogenes, including c-*myc*, c-*myb*, c-*ras*^{Ki}, c-*ras*^{Ha}, c-*abl*, and c-*erb* B oncogenes and of N-*myc*, have been reported in a number of human cancer cell lines (*5–7*) and in a few fresh human tumors (*8–11*).

Since most analyses have been performed with cultured tumor cells, it has been unclear how frequently alterations of cellular oncogenes occur in vivo. Furthermore, the possibility that similar alterations occur in various normal tissues has not been formally excluded. To obtain information on these points we examined 101 fresh human malignant tumors and 3 benign tumors representing 21 different histologic types, including 71 carcinomas, 9 sarcomas, 18 leukemias, and 3 lymphomas (Table 1). In 72 instances it was possible to obtain simultaneously normal tissues from the same patients, and in 64 of these the normal tissues were homologous with the cancers (such as colon cancer and normal colonic mucosa). DNA from these 176 samples was analyzed for alterations in proto-oncogenes with Southern blot hybridization (*12*) and 11 probes hybridizing either with nine different cellular oncogenes or with human β-globin (Table 2)(*6, 13–22*). Alterations were frequently found in c-*myc*, c-*ras*^{Ha}, and c-*myb*, and rarely in c-*ras*^{Ki} in tumors in vivo (Table 1). In contrast, no alterations in c-*fos*, c-*fes*, c-*abl*, N-*ras*, or c-*mos* were observed in the same samples, and no abnormalities of any of the nine proto-oncogenes in DNA specimens from normal tissues. Oncogene alterations appeared to be correlated with tumor behavior.

Amplification of the c-*myc* oncogene has been observed in several human cancer cell lines (*5–7*) and fresh tumors (*8–10*). The DNA was digested with Eco RI and hybridized (*12*) with a c-*myc*–specific probe and a β-globin probe (Fig. 1A). The single-copy β-globin gene served as an internal control for the amount of DNA transferred to the filters, and was used to estimate the copy number of the c-*myc* oncogene in normal and tumor tissues. In all DNA's tested, the human c-*myc* probe detected a 12.5-kb Eco RI fragment that contained the whole germline or non-rearranged human c-*myc* gene sequence (*23, 24*). The human β-globin probe detected a 3.1-kb Eco RI fragment that is at the 3′ end of the β-globin gene (*22*). The intensity of the β-globin signal was similar among 176 samples of normal

Table 1. Alterations in proto-oncogenes in human cancers.

Tumors	No.	Ratio of number with alterations to number studied			
		Amplified c-*myc*	Deleted c-*ras*^{Ha}	Deleted c-*myb*	Amplified c-*ras*^{Ki}
All types	101	10/101 (10%)	6/33 (18%)	4/35 (11%)	1/101 (1%)
Epithelial* (carcinomas)	71	8/71 (11%)	5/29 (17%)	3/33 (9%)	1/71 (1%)
Sarcomas†	9	2/9 (22%)	1/4 (25%)	1/1 (100%)	0/9 (0)
Leukemia‡ and lymphomas	21	0/21 (0)		0/1 (0)	0/21 (0)
Benign§	3	0/3 (0)	0/3 (0)	0/1 (0)	0/3 (0)
Primary‖	64	7/64 (11%)	4/26 (15%)	3/27 (11%)	0/64 (0)
Metastatic‖	16	3/16 (19%)	2/7 (29%)	1/8 (13%)	1/16 (6%)
None (normal tissue)	72	0/72 (0)	0/36 (0)	0/35 (0)	0/72 (0)

*Numbers of individual tumors are squamous of head and neck (or), skin (7), lung (3), esophagus (1), stomach (9), colon-rectal (32), kidney (4), breast (10), ovary (5). †Osteogenic (2), chondrosarcoma (1), soft tissue sarcomas (6). ‡Chronic myelocytic leukemia (8), chronic lymphocytic leukemia (6), acute myelocytic leukemia (3), acute lymphocytic leukemia (1), malignant lymphoma (3). §Colonic polyp (1), desmoid tumor (2). ‖Excluding leukemias and lymphomas.

Jun Yokota, Yasuko Tsunetsugu-Yokota, Carol Le Fevre, Martin J. Cline, Department of Medicine, Center for Health Sciences, University of California at Los Angeles, Los Angeles 90024.
Hector Battifora, Department of Surgical Pathology, City of Hope–National Medical Center, Duarte, CA 91010.

and tumor tissues. The intensity of the c-*myc* signal was also similar among the 72 samples of normal tissue; however, we observed ten tumor samples with a more intense signal corresponding to the 12.5-kb c-*myc* fragment, indicating an increase in the copy number of the c-*myc* gene (Table 1 and Figs. 1A and 4). Quantitative comparison revealed a three- to eightfold amplification in tumors. The tumors demonstrating amplification were squamous carcinomas of head and neck (2 of 7), bronchioloalveolar carcinoma of lung (1 of 2), carcinomas of colon and rectum (2 of 32), breast (2 of 10), and ovary (1 of 5), an osteogenic sarcoma (1 of 2), and a rhabdomyosarcoma (1 of 1).

Amplification of c-*myc* was not seen in any of the 21 hematologic malignancies examined. In an independent study of two patients with acute promyelocyte leukemia and six with acute myeloid leukemia we also failed to detect c-*myc* amplification (*25*). By contrast, in solid tumors amplification of c-*myc* occurred in 11 percent (7/64) of primary tumors and in 19 percent (3/16) of metastatic tumors. The extent of amplification was relatively higher in metastatic tumors (five- to eightfold) than in primary tumors (three- to fivefold), suggesting a correlation between c-*myc* amplification and tumor metastasis or progression. In addition, no c-*myc* bands other than the germline 12.5-kb band were seen, suggesting that gross rearrangements of the c-*myc* gene are not as common in other malignancies as in Burkitt's lymphoma (*2*).

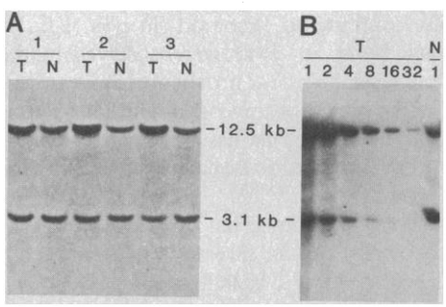

Fig. 1. Amplified c-*myc* in human cancers. Tumors and homologous normal tissues obtained at surgery and kept at −70°C were chilled in liquid nitrogen, ground to powder, disrupted in isotonic buffer, and centrifuged. The pellet was digested with Pronase and purified on a CsCl gradient. Twenty micrograms of DNA were digested with Eco RI, subjected to electrophoresis through 0.8 percent agarose, denatured, and transferred to nitrocellulose filters (*12*). The filters were hybridized with ^{32}P-labeled (nick-translated) c-*myc* and β-globin probes under stringent conditions, washed, dried, and exposed to Kodak XAR-5 film. (A) DNA from tumors (T) and homologous normal (N) tissues were from patients with colon adenocarcinoma (A1), bronchioloalveolar carcinoma of lung (A2), and osteogenic sarcoma (A3). The c-*myc* signal (12.5 kb) was more intense in tumor than in normal DNA's. The intensity of the β-globin signal (3.1 kb) was similar in normal and tumor tissues. (B) DNA from a bone marrow metastasis of a squamous cell carcinoma of tonsil showed an eightfold amplification of the c-*myc* gene. The extent of amplification in the tumor was estimated by densitometric comparison of the signals in tumor and normal tissue DNA and confirmed by serially diluting DNA's to obtain a hybridization signal of about single-copy intensity, equal to that seen in normal DNA (N1).

Those primary tumors showing c-*myc* amplification were all aggressive and disseminated at the time of initial surgery and DNA analysis. For example, the primary breast cancers were large (8 and 10 cm in diameter) and had positive axillary lymph nodes (2 of 14 and 16 of 26) at initial resection. The patient with an ovarian tumor had widespread metastases and died within a short time of surgery, as did a 61-year-old man with disseminated poorly differentiated squamous cell carcinoma of the tonsil, and a 6-year-old boy with disseminated rhabdomyosarcoma.

The c-*ras*^{Ha} oncogene has a Bam HI restriction fragment length polymorphism (*26*). DNA sequence data suggest that the basis for this polymorphism is a region of tandemly repeated nucleotides linked closely to the 3′ end of *ras*^{Ha} coding sequences (*27*). DNA's digested with Bam HI, blotted, and hybridized to the c-*ras*^{Ha} probe have either a single band (indicating homozygosity) or a doublet structure (indicating heterozygosity) of size 6.6 to 8.7 kb (Fig. 2A). None of the 176 tumor or normal DNA's had detectable amplification of the c-*ras*^{Ha} oncogene; however, apparent loss of one c-*ras*^{Ha} allele occurred in some cancers.

We compared the c-*ras*^{Ha} Bam HI restriction pattern in tumors and normal tissues in 36 heterozygous patients. Three had benign tumors and 33 had malignant disease. The intensity of the two allelic bands was the same in the 36 heterozygous normal tissue DNA samples, in 3 DNA's from benign tumors, and in 27 heterozygous DNA samples from malignant tumors; however, the intensity of one band (arrow in Fig. 2B) was less than the other band in six malignant tumor samples (Figs. 2 and 4). This observation was consistent with loss of one c-*ras*^{Ha} allele, and could not readily be explained by a restriction site mutation, since no anomalous hybridizing bands were observed. *Ras*^{Ha} deletions were observed in carcinomas of colon, lung, breast, and ovary and in one sarcoma (malignant fibroxanthoma). This phenomenon was almost twice as frequent in metastases (29 percent) as in primary tumors (15 percent). We could not perform a similar analysis in leukemic cells because of a lack of available homologous normal tissues; however, the relative frequency of detectable heterozygotes (12 of 18 leukemias) with equally hybridizing alleles suggests that loss of a c-*ras*^{Ha} allele is not common in leukemia (Fig. 2A).

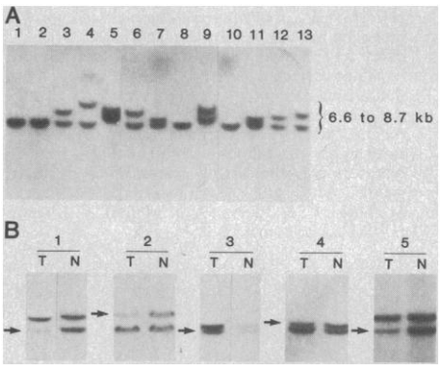

Fig. 2. (A) Bam HI restriction fragment length polymorphism of the c-*ras*^{Ha} locus: DNA was obtained from leukemic cells and purified by proteinase K digestion and phenol extraction. DNA's digested with Bam HI, blot transferred, and hybridized to a c-*ras*^{Ha} probe had either a single band or a doublet structure of size 6.6 to 8.7 kb. The intensity of the two allelic bands was the same in nine heterozygous DNA's. (B) Allelic deletion of c-*ras*^{Ha} locus in five tumor samples. Tumor (T) and normal (N) DNA was obtained from patients with colon cancer (B1), breast cancer (B2), fibroxanthoma (B3), adenocarcinoma of lung (B4), and ovarian cancer (B5). The intensity of one allelic band (arrow) was decreased in tumor DNA's, while both bands were of similar intensity in normal tissues, suggesting a partial loss of one allele of the c-*ras*^{Ha} locus in tumors.

We analyzed 176 tumor and normal DNA's digested with Hind III and hybridized to the c-*myb*–specific probe. Two bands of 7.1 and 4.3 kb were found in all samples (Fig. 3A), and the intensity was similar in all. We concluded that the c-*myb* gene was neither detectably amplified nor rearranged in these tumors and normal tissues.

Polymorphisms were not found in DNA's digested with Pst I, Bam HI, Sac I, and Xba I. Of three different Eco RI restriction fragment patterns (Fig. 3B), the first had two bands of 2.8 and 2.6 kb, the second had four bands of 2.8, 2.6, 1.55, and 1.05 kb, and the third had three bands of 2.8, 1.55, and 1.05 kb. The second and third Eco RI restriction patterns are probably based on an additional Eco RI site within the 2.6-kb fragment that generates 1.55- and 1.05-kb bands instead of a 2.6-kb band.

In our panel of tumors and normal tissues, 36 pairs had four *myb* bands after Eco RI digestion, indicating heterozygosity at the c-*myb* locus on chromosome 6. Thirty-seven had two or three bands, indicating homozygosity. In 4 of the 35 heterozygous pairs in which the tumors were malignant, the intensity of either the 2.6-kb band, or of both the 1.55- and 1.05-kb bands, was less

in tumor DNA than in the corresponding normal DNA (Fig. 3C). This alteration might result from loss of one allele, or a mutation at an internal restriction site in some tumor cells. It suggests instability of the c-*myb* locus. An altered allelic ratio was observed in a bronchioloalveolar carcinoma (a rare lung tumor), a breast cancer, a colon cancer, and an osteogenic sarcoma. Apparent allelic deletion was not found in any of 36 normal tissues or in a benign colonic polyp. Apparent deletion was equally common in primary and metastatic tumors (11 to 13 percent; Table 1), but might be more frequent in sarcomas (1 of 1 examined) and bronchioloalveolar carcinomas (1 of 1 examined) than other tumors.

An interesting observation was an altered allelic ratio of c-*myb* in a metastasis of a breast cancer that was not present in either the primary tumor or in the homologous normal breast tissue (Fig. 3C, panel 4). This observation suggests that allelic alterations may occur concomitantly with either tumor progression or metastasis.

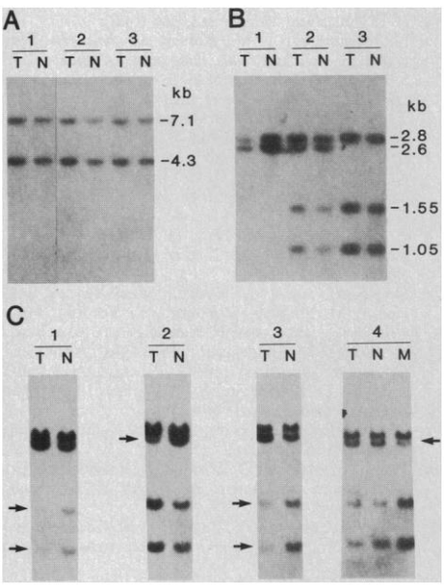

Fig. 3. Analysis of the c-*myb* oncogene. Ten micrograms of DNA were digested with Hind III (A) or Eco RI (B), blot transferred, and hybridized with a c-*myb*–specific probe. Tumor (lanes T) and normal (lanes N) tissue DNA's were obtained from patients with desmoid tumor (A1), colon adenocarcinoma (A2), and gastric adenocarcinoma (A3). Two bands of 7.1 kb and 4.3 kb were found in all samples digested with Hind III. Three different restriction patterns were observed in samples digested with Eco RI. The first (B1) had two bands of 2.8 and 2.6 kb; the second (B2) had four bands of 2.8, 2.6, 1.55, and 1.05 kb; and the third (B3) had three bands of 2.8, 1.55, and 1.05 kb. (C) DNA was obtained from colon adenocarcinoma (C1), bronchioloalveolar carcinoma of the lung (C2), osteogenic sarcoma (C3), and breast cancer (C4), digested with Eco RI, and hybridized with a c-*myb*–specific probe. The intensity of either the 2.6-kb band, or of both the 1.55- and 1.05-kb bands (arrows), was less in the primary tumor DNA (T) or metastatic tumor DNA (M) than in the corresponding normal DNA (N).

Of the tumors examined, only one had multiple oncogene abnormalities. This papillary cystadenocarcinoma of the ovary was unusual in its genetic composition and was unusually aggressive clinically. It came from a 51-year-old woman, diagnosed as having bilateral ovarian tumors in November 1982. She was treated with extensive surgery, radiation therapy, cyclophosphamide, vincristine, doxorubicin, and *cis*-platinum. A biopsy was performed in July 1984 and the tumor DNA was analyzed. Three months later the patient died; the tumor had spread to the stomach, small intestine, colon, diaphragm, spleen, kidney, adrenal, pleurae, pericardium, pancreas, lymph nodes, lungs, breasts, and skin. Analysis of DNA from an omental metastasis revealed three abnormalities: (i) a fivefold amplification of c-*myc*; (ii) a partial deletion of one allele of c-ras^{Ha}; and (iii) an approximately fourfold amplification of c-ras^{Ki} (Fig. 4). These alterations were absent from normal tissues and in c-*myb*, c-*abl*, c-*fos*, c-*fes*, N-*ras*, or c-*mos* in the tumor DNA (Fig. 4). The abnormalities solely in the tumor DNA suggest that the specific oncogene changes were associated with malignancy and not the chemotherapy and irradiation.

Amplification of c-*myc* and apparent loss of one allele of c-ras^{Ha} or c-*myb* were relatively common in malignant tumors, whereas amplification or gross rearrangements of c-*fos*, N-*ras*, c-*mos*, c-*abl*, and c-*fes* were not detected. Our principal observations were the following. (i) Amplification of c-*myc* was rare in leukemias or lymphomas and was relatively common in epithelial tumors and sarcomas (11 to 22 percent). (ii) Amplification of c-*myc* appeared to correlate with aggressive primary tumors and with tumor progression. (iii) Apparent loss of one allele of either c-*myb* or c-ras^{Ha} was relatively common in solid tumors (11 to 15 percent) and may be more frequent in sarcomas than carcinomas. (iv) Allelic alteration at the c-*myb* locus occurred in a tumor metastasis, but was absent in the normal tissue and primary tumor, suggesting a correlation with progression of malignant disease. (v) Loss of a c-ras^{Ha} allele occurred more frequently in metastatic tumors (29 percent) than in primary tumors (15 percent). (vi) Rare tumors have abnormalities of more than one proto-oncogene (amplification of c-ras^{Ki} and c-*myc* and loss of an allele of c-ras^{Ha} in an aggressive ovarian carcinoma). (vii) Alterations of oncogenes were not detected in over 70 DNA samples from normal tissues.

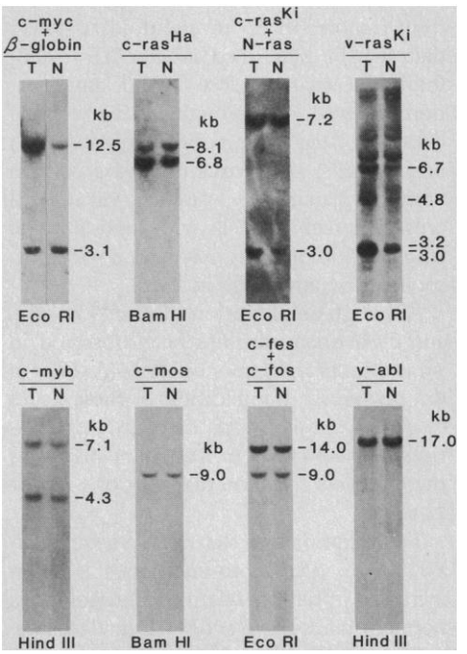

Fig. 4. Multiple proto-oncogene alterations in a metastatic papillary cystadenocarcinoma of the ovary. Tumor (T) and normal (N) tissue DNA were digested with Eco RI, Hind III, or Bam HI and hybridized with one or two probes under high-stringency conditions or, in the case of v-ras^{Ki} and v-*abl*, low-stringency conditions. A fivefold amplification of c-*myc*, a partial deletion of one allele (8.1-kb band) of c-ras^{Ha}, and a fourfold amplification of c-ras^{Ki} were observed in the tumor. Since the c-ras^{Ki} probe detects only a small portion of the proto-oncogene, a v-ras^{Ki} probe (pHiHi3) was also used to confirm amplification.

Previously, amplification of the c-*myc* oncogene has been observed in cell lines derived from lung, stomach, breast, and colon cancers, and neuroblastoma (*5*–*7*); it has also been observed in primary tumors from single patients with acute promyelocytic leukemia (*8*), chronic myelocytic leukemia (*10*), and Burkitt's lymphoma (*9*). We observed amplification in about 11 percent of solid tumors, but not in any hematological malignancy. Rothberg *et al.* observed amplification of the c-*myc* gene in only one case of Burkitt's lymphoma among 106 cases of fresh leukemias and lymphomas (*9*). Therefore, the type of amplification observed in the donor of the HL-60 promyelocytic cell line (*8*) must be a rare phenomenon. Our data further suggest that c-*myc* amplification is associated with aggressive primary or disseminated advanced epithelial cancers and sarcomas. A similar phenomenon has been observed with amplification of the N-*myc* gene in disseminated or clinically aggressive neuroblastomas (*28*) and other neuroendocrine tumors.

The extent of c-*myc* gene amplification in

fresh tumors (three- to eightfold) is lower than that in established cell lines (5- to 50-fold). The relatively low level of amplification observed in vivo might be the result of dilution of the tumor cell population with normal cells, since tumors always contain normal inflammatory, vascular, or stromal cells. Alternatively, cells with high levels of amplification of c-*myc* may have a particular proliferative advantage in vitro.

Although amplification of c-*ras*^{Ha}, c-*myb*, and c-*abl* oncogenes has been observed in some tumors and tumor cell lines (*5–10*), we did not detect amplification of these genes or of c-*mos*, c-*fes*, N-*ras*, or c-*fos*. Amplification of these genes in malignant disease is therefore less common than that of c-*myc* or N-*myc*.

Transcription of the c-*myc*, c-*ras*^{Ha}, c-*ras*^{Ki}, and c-*fos* proto-oncogenes is often increased in tumors relative to homologous normal tissues in vivo (*29*). Our data suggest that this differential expression is unlikely to be the consequence of gene amplification in most cases. We did not repeat the determination of gene expression because of the difficulty of analyzing a cell type homologous to cancer cells in the heterogeneous cell populations that comprise normal tissues; for instance, colon cancer is primarily epithelial, whereas normal colon is composed of many cell types.

Loss of one allele of the c-*ras*^{Ha} oncogene occurred in 18 percent of tumors and of the c-*myb* oncogene in 11 percent of tumors but not in normal cells of the same individuals. Recently Krontiris *et al.* reported that the c-*ras*^{Ha} locus was occasionally involved in loss of one allele in tumors (*4*). Our results confirm their observations and indicate that one allele of this gene is deleted in a wide variety of tumor types in vivo. Apparent deletions also occur at the c-*myb* locus in some tumors, although we could not formally exclude the possibility of a site-specific mutation that generates an Eco RI restriction site in some tumors and deletes it in others. We did not detect complete loss of a c-*ras*^{Ha} allele or of a c-*myb* allele in any case in this study; however, apparent incompleteness of loss might result either from dilution of the tumor cell population by normal cells; or from heterogeneity of the tumor cell population.

The apparent deletions involving c-*ras*^{Ha} or c-*myb* loci may reflect loss of chromosomes or parts of chromosomes during the proliferation of cancer cells. These changes may be random or may be related to the disease. Favoring the random-loss concept are observations in 24 melanoma cell lines indicating that homozygosity or "hemizygosity" of a variety of genes is common and evidently not restricted to specific chromosomes (*30*). Other analyses support the concept that loss of normal regulatory genes (anti-oncogenes) may be associated with the development of malignancy (*31*). These genes might act by regulating an important step in normal cellular differentiation, or by modulating the activity of other potentially carcinogenic genes. Loss or functional inactivation of such suppressor genes might allow tumor development or progression. Cytogenetic analysis of hybrids between normal and malignant cells with suppression of the malignant phenotype are consistent with the existence of suppressor genes (*31*). The best studied clinical example of loss of a potential suppressor gene involves the Rb-1 gene of sporadic and hereditary retinoblastomas. Cavenee and colleagues have discussed potential mechanisms of chromosomal rearrangements in these tumors (*32*).

Our observations indicate that there may be correlations between specific changes in proto-oncogenes and the clinical behavior of tumors. They suggest that amplification of c-*myc* correlates with aggressive primary tumors and tumor spread, that allelic alterations of c-*myb* occurs with tumor progression, that deletions of c-*ras*^{Ha} are more frequent as tumors metastasize; and that multiple oncogene abnormalities are found in particularly aggressive and widely disseminated cancers. These observations are consistent with the concept of a clonal evolution of cancer cell populations that generates variants with selective growth advantages in vivo (*1, 31*).

Table 2. Cloned genes used in the analysis of tumor DNA.

Genes	Size of probe DNA (kb)	Enzyme cleaving tumor DNA	Size of genomic fragment detected (kb)	References
v-*abl*	Bgl II–Bgl II (2.4)	Hind III	17.0	(*13*)
Human c-*myc*	Pst I–Pst I (0.4)	Eco RI	12.5	(*6*)
Human c-*ras*^{Ha}	Sac I–Sac I (3.0)	Bam HI	6.6 to 8.7*	(*14*)
Human c-*fos*	Pst I–Pvu II (0.66)	Eco RI	9.0	(*15*)
Human c-*fes*	Sac I–Sac I (2.5)	Eco RI	14.0	(*16*)
		Bam HI	4.2	
Human N-*ras*	Pvu II–Pvu II (1.5)	Eco RI	7.2	(*17*)
Human c-*ras*^{Ki}	Eco RI–Hind III (0.64)	Eco RI	3.0	(*18*)
v-*ras*^{Ki}	Eco RI–Eco RI (1.0)	Eco RI	6.7, 4.8, 3.2, 3.0	(*19*)
Human c-*mos*	Eco RI–Eco RI (2.75)	Bam HI	9.0	(*20*)
		Eco RI	2.75	
Human c-*myb* (cDNA)	Sac I–Hha I (0.75)	Hind III	7.1, 4.3	(*21*)
		Eco RI	2.8 (2.6* or 1.55,* 1.05*)	
Human β-globin	Eco RI–Pst I (0.8)	Eco RI	3.1	(*22*)

*Restriction fragment length polymorphism.

REFERENCES AND NOTES

1. P. H. Duesberg, *Science* **228**, 669 (1985).
2. N. Heisterkamp *et al.*, *Nature (London)* **306**, 239 (1983); A. de Klein *et al.*, *ibid.* 300, 765 (1982).
3. S. H. Orkin, D. S. Goldman, S. E. Sallan, *ibid.* **309**, 172 (1984); A. D. Reeve *et al.*, *ibid.*, p. 174.
4. T. G. Krontiris, N. A. DiMartino, M. Colb, D. R. Parkinson, *ibid.* **313**, 369 (1985).
5. S. Collins and M. Groudine, *ibid.* **298**, 679 (1982); K. Alitalo, M. Schwab, C. C. Lin, H. E. Varmus, J. M. Bishop, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 1701 (1983).
6. C. D. Little, M. M. Nau, D. N. Carney, A. F. Gazdar, J. D. Minna, *Nature (London)* **306**, 194 (1983).
7. D. Kozbor and C. M. Croce, *Cancer Res.* **44**, 438 (1984); M. Shibuya, J. Yokota, Y. Veyama, *Mol. Cell. Biol.* **5**, 414 (1985); F. Nakasato *et al.*, *Gann* **75**, 737 (1984); K. Alitalo *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **81**, 4534 (1984); P.-G. Pelicci, L. Lanfrancone, M. D. Brathwaite, S. R. Wolman, R. Dalla-Favera, *Science* **224**, 1117 (1984).
8. R. Dalla-Favera, F. Wong-Staal, R. Gallo, *Nature (London)* **299**, 61 (1982).
9. P. G. Rothberg, M. D. Erisman, R. E. Diehl, M. G. Rovigatti, S. M. Astrin, *Mol. Cell. Biol.* **4**, 1096 (1984).
10. D. M. McCarthy, F. V. Rassool, J. M. Goldman, S. V. Graham, G. D. Birnie, *Lancet* **1984-II**, 1362 (1984).
11. K. Hayashi, T. K. Kakizoe, T. Sugimura, *Gann* **74**, 798 (1983); M. Schwab *et al.*, *Nature (London)* **305**, 245 (1983); W. H. Lee, A. L. Murphee, W. F. Benedict, *ibid.* **309**, 458 (1984).
12. E. M. Southern, *J. Mol. Biol.* **98**, 503 (1975).
13. J. R. Selden *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 7289 (1983).
14. S. Pulciani, E. Santos, A. V. Lauver, L. K. Long, M. Barbacid, *J. Cell. Biochem.* **20**, 51 (1982).
15. T. Curran, W. P. MacConnell, F. van Straaten, I. M. Verma, *Mol. Cell. Biol.* **3**, 914 (1983).
16. M. D. Trus, J. G. Sodroski, W. A. Haseltine, *J. Biol. Chem.* **257**, 2730 (1982).
17. M. J. Murray *et al.*, *Cell* **33**, 749 (1983).
18. M. S. McCoy *et al.*, *Nature (London)* **302**, 79 (1983).
19. R. W. Ellis *et al.*, *ibid.* **292**, 506 (1981).
20. R. Watson, M. Oskarsson, G. F. Vande Woude, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 4078 (1982).
21. D. J. Slamon *et al.*, in preparation.
22. R. M. Lawn, A. Efstratiadis, C. O'Connell, T. Maniatis, *Cell* **21**, 647 (1980).
23. R. Taub *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 7837 (1982).
24. R. Dalla-Favera, S. Martinotti, R. C. Gallo, J. Erikson, C. M. Croce, *Science* **219**, 963 (1983).
25. D. J. Slamon, M. J. Cline, P. Koeffler, unpublished data.
26. M. Goldfarb, K. Shimizu, M. Perucho, M. Wigler, *Nature (London)* **296**, 404 (1982).

Nature (London) **296**, 404 (1982).
27. D. J. Capon, E. Y. Chen, A. D. Levinson, P. H. Seeburg, D. V. Goeddel, *ibid.* **302**, 33 (1983).
28. G. M. Brodeur, R. C. Seeger, M. Schwab, H. E. Varmus, J. M. Bishop, *Science* **224**, 1121 (1984).
29. D. J. Slamon, J. B. deKernion, I. M. Verma, M. J. Cline, *ibid.*, p. 256.
30. N. C. Dracopoli, A. N. Houghton, L. J. Old, *Proc. Natl. Acad. Sci. U.S.A.* **82**, 1470 (1985).
31. G. Klein and E. Klein, *Nature (London)* **315**, 190 (1985).
32. W. K. Cavenee *et al.*, *ibid.* **305**, 779 (1983); W. K. Cavenee, M. F. Hansen, M. Nordenskjold, E. Kock, I. Maumenee, J. A. Squire, R. A. Phillips, B. L. Gallie, *Science* **228**, 501 (1985).
33. Supported by USPHS grant CA15619, grant DRG-030 from the Damon-Runyon Foundation (J.Y.), and a grant from Triton Biosciences.

31 July 1985; accepted 9 October 1985

Interferon and c-*ets*-1 Genes in the Translocation (9;11)(p22;q23) in Human Acute Monocytic Leukemia

MANUEL O. DIAZ,* MICHELLE M. LE BEAU, PAULA PITHA, JANET D. ROWLEY

Gene probes for interferons α and β_1 and v-*ets* were hybridized to metaphase chromosomes from three patients with acute monocytic leukemia who had a chromosomal translocation, t(9;11)(p22;q23). The break in the short arm of chromosome 9 split the interferon genes, and the interferon-β_1 gene was translocated to chromosome 11. The c-*ets*-1 gene was translocated from chromosome 11 to the short arm of chromosome 9 adjacent to the interferon genes. No DNA rearrangement was observed when these probes were hybridized to genomic DNA from leukemic cells of two of the patients. The results suggest that the juxtaposition of the interferon and c-*ets*-1 genes may be involved in the pathogenesis of human monocytic leukemia.

SPECIFIC CHROMOSOMAL ABNORMALities are associated with defined types of leukemia (*1*). In two of these abnormalities, the t(8;14)(q24;q32) in Burkitt's lymphoma (BL) and the t(9;22)(q34;q11) in chronic myelogenous leukemia (CML), cellular proto-oncogenes associated with one of the chromosomal breakpoints (*2, 3*) are linked to another cellular gene. The immunoglobulin (Ig) genes are expressed specifically in B cells, which are the neoplastic cells in BL; c-*myc* is under the control of regulatory mechanisms that are related to the Ig genes. In CML, the *bcr* and c-*abl* genes are fused in the Philadelphia chromosome, resulting in transcription of a chimeric messenger RNA (mRNA) under the control of the *bcr* promoter.

Abnormalities of chromosome 11 involving band q23 are observed frequently in acute monocytic leukemia (AMoL), particularly in children (*4*). A common rearrangement, noted in about 10 percent of patients with AMoL, is the translocation (9;11)(p22;q23); the precise breakpoint in 9p, however, is a subject of disagreement. Analysis of trypsin-Giemsa–banded metaphase cells of our patients has indicated that all of band 9p21 remains on chromosome 9, and thus we have designated the breakpoint as 9p22. Other abnormalities observed in patients with AMoL include the t(11;19)(q23;p13) and del(11)(q23). Rearrangements involving 11q23 have also been reported in other hematologic malignant diseases. In particular, the t(4;11)(q21;q23) has been observed in patients with acute lymphoblastic leukemia (*5*), and leukemic cells with this abnormality may have cell surface markers characteristic of the monocyte lineage (*6*). The breakpoint in the t(9;11) at band 9p22 is also involved in other structural rearrangements. For example, deletions and translocations of the short arm of chromosome 9 are associated with a distinct subset of acute lymphoblastic leukemia (*7*).

Two sets of cellular genes have been localized to the vicinity of the breakpoints in the t(9;11) by in situ chromosomal hybridization. First, the cellular proto-oncogene c-*ets*-1 has been assigned to bands 11q23-q24 (*8*). The v-*ets* oncogene contains sequences that are homologous to cellular DNA located on chromosomes 11 and 21 (*9*). Two oncogenes, v-*ets* and v-*myb*, are transduced by the avian retrovirus E26 (*10*). This retrovirus causes a monoblastic neoplastic proliferation (myeloblastosis) as well as erythroblastosis in chickens (*10*).

Second, a cluster of interferon (IFN) genes, which include the family of IFN-α genes and the IFN-β_1 gene, has been assigned to the short arm of chromosome 9 at bands 9p13-p24 (*11*). The IFN-α and IFN-β_1 genes belong to a group of eukaryotic genes that encode polypeptides with antiviral and anticellular properties. These genes are not spliced, and their expression can be induced by viral infection in a large variety of cells (*12*). The IFN-α genes are a family of at least 17 closely related genes (*13*) that are clustered on the short arm of chromosome 9 (*11, 14*). Subsets of up to three IFN-α genes arranged in tandem have been isolated on one or a few overlapping genomic clones (*13*). Some of the genes are more closely linked than others, with distances ranging from 3.8 to 18 kilobases (kb) (*13*). The average distance between these linked genes is 10 kb. A second family of IFN-α–like sequences is interspersed in tandem arrangement between the IFN-α genes (*15*). Judging from the linkage disequilibrium of five restriction fragment length polymor-

Table 1. In situ hybridization of IFN and v-*ets* probes to metaphase cells with a t(9;11). The probes were prepared by nick translation of the plasmid DNA's with tritiated deoxynucleoside triphosphates to specific activities of about 3×10^7 dpm/μg: pR-78 contains human IFN-α_1 complementary DNA (cDNA) (*24*), pHFβ cDNA contains human IFN-β_1 cDNA (*25*), and p-ets-BB contains v-*ets* sequences derived from the virus E26 (*21*). In situ hybridization was performed as described previously (*26*). Metaphase cells were hybridized at 20 or 40 ng of probe per milliliter of hybridization mixture.

Probe	Patient	Number of labeled sites (number of metaphase cells analyzed)	Number of labeled sites (%)			
			Normal 9	t(9p)	Normal 11	t(11q)
IFN-β_1	1	128 (100)	15 (11.7)*	1 (0.8)	3 (2.3)	10 (7.8)*
	2	74 (50)	6 (8)*	2 (2.7)	2 (2.7)	6 (8)*
IFN-α_1	2	267 (82)	22 (8.2)*	20 (7.5)*	8 (3)	8 (3)
	3	52 (20)	6 (11.5)*	8 (13.5)*	0	3 (5.8)
v-*ets*	2	96 (60)	4 (4.2)	8 (8.3)*	11 (11.5)*	2 (2.1)
	3	50 (20)	1 (2)	6 (12)*	7 (14)*	0

*χ^2 value corresponds to $P < 0.0005$. The χ^2 analysis tests the hypothesis that labeling is random over all chromosomes.

M. O. Diaz, M. M. Le Beau, J. D. Rowley, Joint Section of Hematology/Oncology, Department of Medicine, University of Chicago, Chicago, IL 60637.
P. Pitha, Oncology Center, Johns Hopkins Hospital, Baltimore, MD 21205.

*Request for reprints should be sent to Box 420.