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RESEARCH ARTICLE

Expression of Cellular Oncogenes in Human Malignancies

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Most acutely transforming RNA tumor viruses contain genomic sequences that appear to be responsible for the induction of neoplasia. These genes have been termed viral oncogenes (v-onc) (*1*). The exact mechanism by which v-onc gene products mediate the transformation of normal to neoplastic cells remains unclear.

Nineteen v-onc genes have been identified and isolated (*2–4*), and DNA sequences homologous to the transforming genes of certain retroviruses have been found in normal untransformed cells (*5, 6*). DNA sequences homologous to most of the known v-onc genes have been identified in a variety of normal uninfected cells, including those of man (*2–7*). These genes—termed cellular oncogenes (c-onc)—appear to have been the evolutionary progenitors of the v-onc genes (*3, 4, 8*). Viral acquisition of the genes is believed to have occurred by recombinational events between the genome of the infecting retrovirus and that of the host cell (*8*).

The structural similarity between c-onc genes and their viral homologs suggests that the former may also possess an oncogenic potential. Three lines of evidence support this idea. First, two cloned c-onc genes, c-*mos* and c-*ras*^{Ha}, when coupled to a retroviral long terminal repeat (LTR) and transfected into NIH 3T3 cells, induce transformation (*9, 10*). Second, the avian leukosis viruses (ALV), which lack an identifiable onco-

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gene, induce malignancy after long latent periods. The mechanism appears to involve insertion of the viral genome adjacent to the c-onc gene (c-*myc*), which is thereby activated (*11*). Third, certain human tumor cell lines and tumors contain genes that induce a transformed phenotype when introduced into mouse 3T3 cells (*12–15*). Several such genes have been identified as the c-onc genes known as c-ras^{Ha} and c-ras^{Ki} (*13–15*).

We have investigated the association between the known c-onc genes and freshly obtained human malignancies. Using the presence of messenger RNA (mRNA) transcripts as a parameter of gene expression, we posed the following questions: Which c-onc genes are expressed in fresh human malignancies? Are there differences in the levels of expression of these oncogenes in tumors and the corresponding normal tissues? Is there a consistent association between tumor type and oncogene expression? Because of difficulties in obtaining undegraded RNA from fresh human tumors other than leukemias (*16*), most initial studies of c-onc gene expression in human neoplasia were restricted to established cell lines (*16–18*). However, phenomena observed in established human malignant cell lines may not always reflect phenomena occurring in tumors in vivo (*19*). We therefore examined 20 different fresh human tumors from 54 patients using DNA-RNA hybridization techniques with 15 viral probes (Table 1) (*20*). Whenever possible, both tumor and homologous normal tissue were obtained simultaneously from the same patient and analyzed for c-onc expression, allowing the patient to serve as his own control.

Expression of Cellular Oncogenes in Tumors

To simplify the presentation of the data, we developed a scale of increasing intensity of hybridization of mRNA to v-onc–specific probes (Fig. 1A). Three patterns of expression were observed.

Abstract. *Cellular oncogenes have been implicated in the induction of malignant transformation in some model systems in vitro and may be related to malignancies in vivo in some vertebrate species. This article describes a study of the expression of 15 cellular oncogenes in fresh human tumors from 54 patients, representing 20 different tumor types. More than one cellular oncogene was transcriptionally active in all of the tumors examined. In 14 patients it was possible to study normal and malignant tissue from the same organ. In many of these patients, the transcriptional activity of certain oncogenes was greater in the malignant than the normal tissue. The cellular* fes *(feline sarcoma) oncogene, not previously known to be transcribed in mammalian tissue, was found to be active in lung and hematopoietic malignancies.*

1) Four c-onc genes, c-*myc*, c-*fos*, c-ras^{Ha}, and c-ras^{Ki}, were expressed in all or nearly all the tumors examined (see Table 2). An example of this pattern is shown in Fig. 1B.

2) Some c-onc genes, such as c-*abl*, c-*fes*, c-*fms*, and c-*myb*, were expressed infrequently. The data for associations between individual genes and specific tumor types revealed some consistent patterns. Sequences related to c-*fes* and c-*myb* were found in nine of ten hematologic malignancies, and c-*fes* was expressed in all four lung malignancies (Table 2). C-*abl*–related sequences were found in only two cases of chronic leukemia and in one case of acute leukemia. Other investigators have described a wider distribution of c-*abl*–related sequences in human tumor and leukemic cell lines as well as some fresh leukemias (*18*). Our finding of a more restricted distribution may have resulted from our use of a v-*abl* probe that does not encompass the kinase domain (Table 1). Others have used probes that do encompass the kinase domain of v-*abl* and, since this domain shares sequences with other oncogene products with tyrosine-specific kinase activity (*21*), it may, by extension, be homologous with cellular tyrosine phosphokinases. C-*fms*–related sequences were found in 18 of 54 tumors with no apparent specific association. Strongest expression of c-*fms* was observed in two breast malignancies, a Hodgkin's lymphoma, a renal cell carcinoma, and in chronic myelogenous leukemia. C-*src*–related sequences were found in leukemic cells of a patient with chronic myelogenous leukemia and in circulating tumor cells of a patient with lymphosarcoma.

3) The third pattern of expression was found with the c-erb^{A}, c-erb^{B}, c-*mos*, c-*rel*, c-*sis*, and c-*yes* genes, in which no gene transcripts were detectable. An example of this pattern is shown in Fig. 1B. This result did not reflect a lack of sequence homology between the viral gene

Table 1. Oncogenes used as probes.

Retroviral source	Viral oncogene	Fragment used as probe	Reference
Avian			
Rous sarcoma virus	v-*src*	Pvu II–Pvu II (0.8 kbp)	*42*
Y-73 sarcoma virus	v-*yes*	Pst I–Pst I (1.2 kbp)	*43*
Avian myelocytomatosis virus (MC-29)	v-*myc*	Pst I–Pst I (1.5 kbp)	*44*
Avian erythroblastosis virus	v-erb^{A}	Pst I–Pst I (≃ 0.5 kbp)	*45*
	v-erb^{B}	Bam HI–Bam HI (≃ 0.6 kbp)	
Avian myeloblastosis virus	v-*myb*	Bam HI–Bam HI (1.0 kbp)	*46*
Avian reticuloendotheliosis virus	v-*rel*	Eco RI–Eco RI (0.8 kbp)	*47*
Murine			
Moloney sarcoma virus	v-*mos*	Xba I–Hind III (1.1 kbp)	*31, 48*
Abelson leukemia virus	v-*abl*	Bgl II–Bgl II (0.7 kbp)	*49*
Harvey sarcoma virus	v-ras^{Ha}	Bgl I–Sal I (0.4 kbp)	*50*
Kirsten sarcoma virus	v-ras^{Ki}	Hinc II–Hinc II (1.0 kbp)	*51*
FBJ osteosarcoma virus	v-*fos*	Pst I–Pvu II (1.0 kbp)	*52*
Feline			
Feline sarcoma virus (GH/ST strain)	v-*fes*	Pst I–Pst I (0.5 kbp)	*53*
Feline sarcoma virus (McDonough strain)	v-*fms*	Pst I–Pst I (1.5 kbp)	*54*
Primate			
Simian sarcoma virus	v-*sis*	Pst I–Xba I (1.0 kbp)	*55*

Table 2. Summary of data on the expression of 15 c-onc genes. Symbols: 0, no expression detected; + to ++++, low to high levels of expression. Transcripts of the following genes were not observed in any of the 54 tumors: erb^{A}, erb^{B}, *mos*, *rel*, *sis*, and *yes*.

Type of tumor	Number of tumor	*abl*	*fes*	*fos*	*fms*	*myb*	*myc*	ras^{Ha}	ras^{Ki}	*src*
					Renal malignant					
Renal cell carcinoma	1	0	0	++	0	0	+++	++	++	0
	2	0	0	+++	0	0	+++	++	++	0
	3	0	0	+++	0	0	+++	++	++	0
	4	0	0	++	0	0	++	++	++	0
	5	0	0	0	0	0	0	0	0	0
	6	0	0	++	+	0	+	+	0	0
	7	0	0	++	++	0	++	++	0	0
	8	0	++	0	++++	0	++	0	++	0
	9	0	+	+++	++	0	+	+	0	0
					Gynecologic malignancies					
Ovarian adenocarcinoma	1	0	0	++++	0	0	+++	++	++	0
	2	0	0	++++	0	0	++++	+++	+++	0
	3	0	0	++	+	0	++	++	+	0
	4	0	0	++++	+	0	++	+	0	0
	5	0	0	++++	+	0	+	++	+	0
	6	0	0	++	+	0	+++	++	0	0
Uterine adenocarcinoma	1	0	0	0	0	0	0	0	0	0
Germ-cell tumor	1	0	0	++	0	0	++	++	++	0
					Gastrointestinal malignancies					
Colon adenocarcinoma	1	0	0	+++	0	0	++	++	++	0
	2	0	0	+++	0	0	++	++	++	0
	3	0	0	+++	0	0	++	++	+	0
	4	0	0	+++	0	0	++	++	+	0
	5	0	0	+++	0	0	+++	+++	+	0
Rectal adenocarcinoma	1	0	0	+++	0	0	+++	++	++	0
	2	0	0	+++	0	0	0	0	0	0
	3	0	0	+++	0	0	++	0	0	0
Cecal adenocarcinoma	1	0	0	+++	+	0	+	+	0	0
Small bowel adenocarcinoma	1	0	0	+++	0	0	++	++	++	0
Pancreatic adenocarcinoma	1	0	0	++	++	0	+	++	0	0
					Lung malignancies					
Carcinoma of lung	1	0	++	++	0	0	+++	+++	+++	0
	2	0	++	++	0	+	++	++	++	0
	3	0	+	++	++	0	++	++	+	0
	4	0	+	+	++	0	+	+	+	0
					Breast malignancies					
Carcinoma of breast	1	0	0	++	0	+	++	++	0	0
	2	0	++	++	+++	++	+++	++	+	0
	3	0	++	++	+++	0	+++	+++	++	0
	4	0	+	++	+++	0	+++	++	++	0
					Sarcomatous malignancies					
Pelvic sarcoma	1	0	0	++	0	0	+	+	+	0
Rhabdomyosarcoma	1	0	0	++	0	0	+++	++	+	0
					Lymphoreticular malignancies					
Thymoma	1	0	0	0	0	0	+	+	0	0
Hodgkin's lymphoma	1	+	+	++	++++	+	++	++	+	0
Non-Hodgkin's lymphoma	1	0	0	++	0	0	+	++	0	0
	2	0	0	++	0	+++	++	++++	+	0
					Hematologic malignancies					
Acute myelogenous leukemia	1	0	++	++++	+	++++	+	++	+++	0
	2	0	++	++	+	++++	+	++	+++	0
	3	0	++	++	0	+++	0	+	++	0
	4	0	++++	0	0	++++	+	0	+	0
	5	0	++++	+	0	++++	+++	+	+	0
	6	0	++++	+	0	+++	++	+	+	0
	7	+	+++	+	0	++++	++	+	++	0
Chronic myelogenous leukemia	1	++	++	0	0	+++	+	+	+	0
	2	+	+++	+++	++++	++	++	++	+	++
Acute lymphocytic leukemia	1	0	+++	++	0	+++	+	+	++	0
	2	0	++	+	0	+++	++	++	+	0
Lymphosarcoma (leukemic phase)	1	0	0	+	0	0	++	+	+	+++

probe and human mRNA, since it was possible to detect homologs of all these probes in human genomic DNA (Fig. 1B and Table 2).

Differential Expression in Normal and Malignant Tissues

To assess whether there was an association between the levels of expression of c-onc gene sequences and neoplasia, we obtained, from 16 of the 54 patients, grossly normal-appearing tissue as well as obviously malignant tissue from the same organ.

We examined the tissues histologically to confirm the presence of at least 80 percent viable-appearing tumor cells in the malignant specimen and the absence of tumor cells in the normal tissue, and then conducted hybridization studies on RNA samples from the tissues. In two of 16 patients the presumed normal tissue was shown by histologic analysis to be infiltrated by tumor (renal cell carcinomas 4 and 7 in Table 2). In 11 of the remaining 14 cases, higher levels of expression of some, but not all, c-onc genes were found in the malignant tissue (Table 3). The levels as determined by comparative densitometry were 2 to 11 times higher than in the corresponding normal tissues. Such increased expression was seen most consistently with transcripts related to c-*myc* and c-ras^{Ha} (10 of 14 cases and 9 of 14 cases, respectively) (Table 3). However, in three cases—an adenocarcinoma of the small bowel and two renal cell carcinomas—the levels of c-onc–related sequences in the tumor and the histologically normal adjacent tissue were similar (Table 3). An example of the microscopic and nucleic acid analyses of a representative case showing differential expression is shown in Figs. 2 and 3.

Quantitation of Cellular Oncogene Transcription

Estimates of the levels of c-onc gene expression in individual tumors were made by spotting tumor RNA on blots with known amounts of viral RNA. In the case of c-*myc*, gradient-purified 70*S* viral RNA was used as the standard. With the use of polyadenylated [poly(A^+)] RNA from renal cell carcinoma tissue, the level of c-*myc*–related sequences was determined to be about 100 pg per microgram of RNA (Fig. 4). With the use of poly(A^+) RNA from the normal renal tissue of the same patient, the level of c-*myc* expression was between 10 and 25 pg per microgram of RNA (Fig. 4). These estimates agree closely with the data obtained by comparative densitometry analyses of the blots [that is, the level of c-*myc*–related sequences in tumor tissue was three to five times higher than in normal tissue (Table 3)]. In an ovarian carcinoma, the level of c-*myc*–related sequences was about 110 pg per microgram of RNA.

In estimating the levels of c-ras^{Ha}– and c-ras^{Ki}–related sequences, RNA from cells transformed with the Harvey and Kirsten murine sarcoma viruses, respectively, were used as standards. In the renal cell carcinoma tissue the level of c-

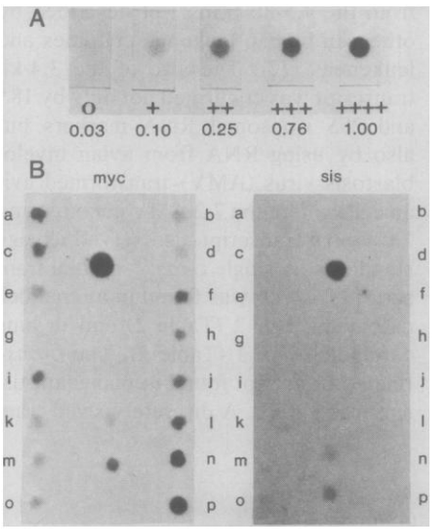


Fig. 1. (A) Scale of intensity of hybridization, 0 to ++++. Numbers indicate relative intensity as determined by soft-laser densitometry. (B) Hybridization of tumor and normal tissue mRNA to v-onc gene probes. Total RNA was extracted by homogenization in guanidine thiocyanate and precipitation in guanidine hydrochloride and ethanol (*57*). To assess the integrity of the RNA, a 5-μg portion of total RNA from each sample subjected to electrophoresis in a 1.1 percent agarose gel and stained with ethidium bromide (0.5 μg/ml in 150 m*M* tris buffer, *p*H 7.4). A 28*S*:18*S* ribosomal RNA ratio of less than 2:1 or fragmentation of either of these species was taken as evidence of degradation, and the sample was not further analyzed. The [poly(A^+)]-rich mRNA fraction was obtained by passage over oligo(dT)-deoxyribose cellulose columns (*58*) and again a 5-μg portion of each sample was subjected to electrophoresis and stained. Sufficient 28*S* and 18*S* ribosomal RNA remained after one passage over oligo(dT) to ascertain integrity of the RNA by the criteria outlined above. Any sample showing evidence of significant degradation was discarded, since negative results could reflect degradation of the RNA rather than an absence of transcripts in the sample. The poly(A^+) RNA was precipitated in 2.5 volumes of ethanol and resuspended in water (2 μg/μl), boiled for 2 to 3 minutes, and quick-cooled on ice; 3 μg was spotted onto nitrocellulose paper previously equilibrated with 20× SSC (1× SSC is 0.15*M* NaCl, 0.015*M* sodium citrate) (*28, 59*). The paper was placed in a vacuum oven at 80°C for 3 to 4 hours, and the blots were prehybridized and hybridized to the various ^{32}P-labeled (nick-translated), molecularly cloned v-onc gene probes listed in Table 1, as described (*28, 29*). Examples of positive hybridization (with v-*myc*) and negative hybridization (v-*sis*) reactions are shown. (a) Renal cell carcinoma No. 1; (b) control kidney No. 1; (c) renal cell carcinoma No. 2; (d) control kidney No. 2; (e) renal cell carcinoma No. 4; (f) control kidney No. 4 containing microscopic tumor infiltrates; (g) control colon No. 1; (h) colon carcinoma No. 1; (i) small bowel carcinoma; (j) normal small bowel; (k) colon carcinoma No. 1; (l) rectal carcinoma; (m) germ-cell tumor; (n) ovarian carcinoma No. 2; (o) lung carcinoma No. 2; (p) lung carcinoma No. 1. Central dots, top to bottom, are complementary DNA of v-onc gene probe, human DNA (1 μg), and human DNA (10 μg). Note that the lack of hybridization of tumor RNA to the v-*sis* probe is not due to nonhomology between the probe and the human c-*sis* homolog, as evidenced by hybridization to human genomic DNA.

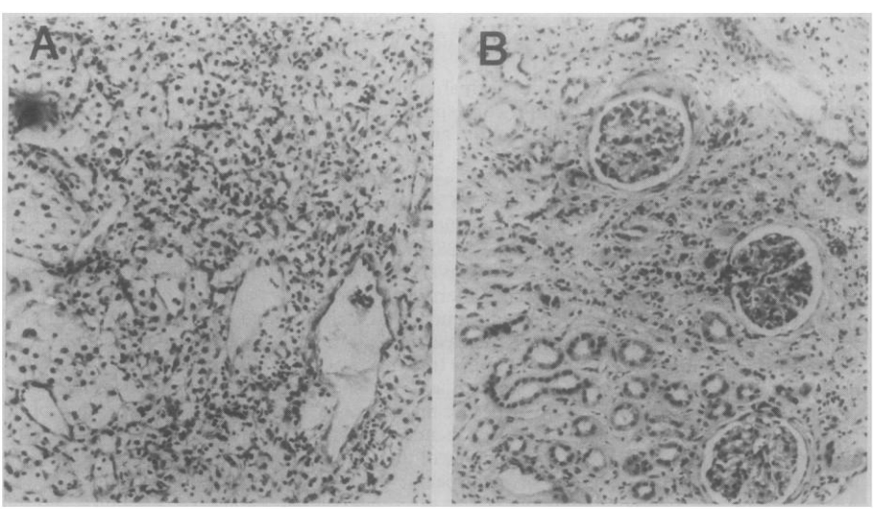
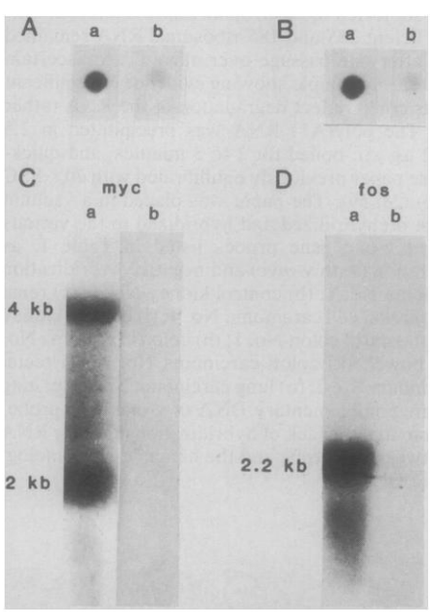


Fig. 2. Photomicrographs of (A) renal cell carcinoma No. 2 and (B) normal kidney adjacent to this carcinoma.

ras^{Ha}–related sequences was about 50 pg/μg. Similar levels were found for c-*ras*^{Ki}–related sequences. These values are only approximations, however, and the methodology may underestimate the actual levels expressed in tissues, since the standards used probably have greater sequence homology with the probes than do the human cellular homologs.

Characterization of Cellular Oncogene Transcripts

Sizes of poly(A$^+$) RNA from tumor tissues were determined by the Northern blot technique (*22*) (Fig. 5). Two c-*myc*–related transcripts of 4.0 and 2.0 kilobases (kb) were found in all tumors examined. There was a small amount of degradation of the mRNA in these hybridization analyses, as evidenced by the tailing seen beneath the c-*myc* bands (Fig. 5). This was probably caused by tissue anoxia during and after surgical removal of the tissue. Transcripts of 2.2 kb related to c-*fos* were demonstrable in almost all of the tumors. A c-*fes* transcript of 2.6 kb was found in poly(A$^+$) RNA from a lung adenocarcinoma and in acute myelogenous leukemia cells. A c-*myb* transcript of 3.4 kb was found in the hematologic malignancies. This differs from the 4.5-kb transcript described by others in human leukemic cell lines and leukemias (*17*). The size of the 3.4-kb transcript was confirmed not only by 18*S* and 28*S* ribosomal RNA markers but also by using RNA from avian myeloblastosis virus (AMV)–transformed avian cells where the 7.2 AMV genomic and 2.0 v-*myb* transcript also served as size standards. A single c-*ras*^{Ha}–related transcript of 1.4 kb was found in a renal cell carcinoma No. 2 (Table 2) and in lung carcinoma No. 1 (Table 2). The c-*fms*–related transcript found in malignant tissue was 3.6 kb. A discretely sized transcript of 4.6 kb related to c-*ras*^{Ki} was detected in a renal cell carcinoma. In a single analysis of the available tissue, no discrete-sized *src* transcripts were found in the lymphosarcoma.



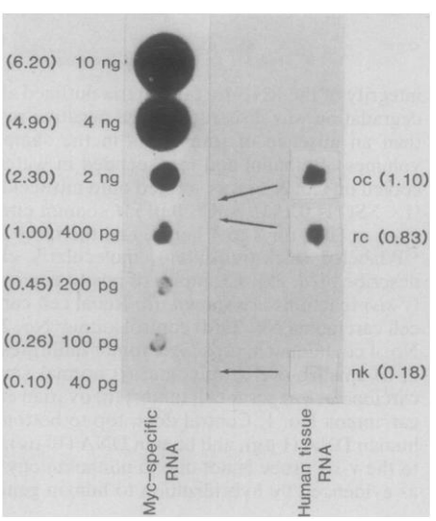


Fig. 3 (left). (A) Dot-blot hybridization of v-*myc* to mRNA of (a) renal tumor and (b) corresponding normal kidney. (B) Dot-blot hybridization of v-*fos*–related transcripts in (a) renal tumor and (b) corresponding normal kidney. (C) Northern analysis of c-*myc*–related transcription of (a) renal tumor and (b) corresponding normal kidney. (D) Northern analysis of c-*fos*–related transcripts of (a) renal tumor and (b) corresponding normal kidney (Northern blotting performed as noted in Fig. 5). Fig. 4 (right). Cellular onc gene expression in malignant and normal-appearing tissue. The sensitivity of the dot hybridization technique was established by spotting serial dilutions of MC-29 viral RNA and hybridization to *myc*-specific probe. The limit of detection of c-*myc*–specific RNA was about 40 pg and was consistent with the range of detection previously published (*28*). Samples giving lesser degrees of hybridization were regarded as negative. Estimates of the levels of c-onc–related RNA in various tumors were obtained by spotting samples on the same filters with either known amounts of gradient-purified viral RNA (in the case of c-*myc*–related sequences) or known amounts of poly(A$^+$)–enriched RNA from virally transformed cells (in the case of *ras*^{Ha}- and *ras*^{Ki}-related sequences). In such transformed cells it has been generally found that the viral RNA constitutes 0.1 to 1 percent of the total cellular message (*60*). Spotting with a v-*myc*–specific probe is shown for comparison. Numbers at left show amount of *myc*-specific message as determined by the percentage of the MC-29 genome that constitutes the *myc* gene. Numbers in parentheses indicate relative absorbances as measured by density scanning of the autoradiogram and are normalized to 1 for the dot at 400 pg. All quantities of *myc*-specific RNA were spotted in a volume of 2 μl. Abbreviations: *oc*, ovarian carcinoma No. 1; *rc*, renal cell carcinoma No. 2; and *nk*, normal renal tissue of the same patient from which renal cell carcinoma No. 2 was obtained. Human tissue RNA dots were made by spotting 4 μg (2 μl) of poly(A$^+$) RNA.

Possible Significance of Cellular Oncogene Messenger RNA in Tumors

Certain aspects of the expression of c-onc genes observed in this survey are of particular interest. First, more than one c-onc gene was transcriptionally active in all of the tumors examined. Although all of the tissue from the solid tumors consisted mostly of tumor cells, specific c-onc transcripts might have arisen from normal inflammatory, vascular, or stromal cells included in the neoplasm. However, completely homogeneous hematopoietic malignancies also demonstrated multiple c-onc gene expression. Furthermore, many human malignant cell lines also express multiple c-onc genes (*16–18, 23*). That multiple c-onc genes are important in malignancy is also suggested by data showing that more than one c-onc gene is needed to transform normal primary embryo fibroblasts into tumorigenic cells (*24*).

A second point of interest is the differential transcriptional activity of c-onc genes in normal and malignant tissue from the same organ of a given patient. Five c-onc genes were expressed at higher levels in some tumors compared with the corresponding normal tissue (Table 3). There was poor correlation between levels of c-onc expression in a given tumor and the ratio of expression in tumor tissue compared to normal tissue (Table 3). This observation held whether one compared expression of different oncogenes in a given tumor or analyzed a single oncogene in different tumor types. This fact underscores the point that while elevated levels of expression of c-onc genes may be found in malignant tissues, they should be interpreted in the context of levels of expression in the corresponding normal tissues. In addition, the significance of differential expression is uncertain, since the comparison may be between different numbers of a given cell type in malignant and normal tissues rather than different levels of transcripts. For example, one adenocarcinoma of the colon consisted of 80 percent viable-appearing tumor cells that were of epithelial origin. In the adjacent normal tissue, the homologous nonmalignant epithelial cells were restricted to the mucosa and represented only 10 to 15 percent of the total cell

population. Techniques that will identify the individual cell types responsible for expression of a given c-onc gene may prove useful in resolving this issue.

A third point demonstrated in the survey is the presence of c-*fes*–related transcripts in some of the malignancies (Table 2). To date, c-*fes*–related transcripts have not been found in any mammalian cell type, although a protein thought to be the product of c-*fes* has been found in some cells (*25*). The highest levels of c-*fes*–related RNA were found in hematopoietic malignancies and in four lung cancers.

Possible Mechanisms of Altered Cellular Oncogene Expression

The c-onc genes are conserved with great fidelity among vertebrates ranging from fish to primates, and indeed some such genes have even been found in invertebrate species (*3, 26*). This conservation probably reflects their role in critical physiologic functions rather than an oncogenic potential, since it seems unlikely that tumor induction provides selective advantage. Many studies have demonstrated the expression of some c-onc genes in normal cells (*6, 27*). We reported that certain oncogenes may play a role during embryonic development of the mouse (*28, 29*). Expression varied with specific c-onc genes in a time-related and tissue-specific pattern. Growth rates exhibited by proliferating embryonic tissues and some malignancies are similar. Thus certain c-onc genes may participate in proliferation or differentiation of normal embryonic and adult tissues and be programmed to become relatively quiescent in terminally differentiated cells, as in the HL-60 model (*17, 18, 23*). Subsequent induction of their expression could be the result of exposure to carcinogenic agents such as radiation, chemicals, or viruses.

Carcinogens might convert normal genes into pathologic genes by inducing higher levels of normal c-onc gene product or by inducing structurally aberrant gene products. There is evidence that both phenomena occur. The concept that higher levels of onc gene products might be important in induction of malignancy initially came from studies of retrovirus-mediated transformation (*30*). In transfection experiments in which the molecularly cloned c-*mos* and c-*ras* genes were linked to LTR's, cell transformation appeared to occur as a result of markedly increased levels of gene product (*10, 14, 31*). In naturally occurring tumors, any of several events could lead to increased gene expression. Amplification of c-*myc* has been described in the human promyelocytic cell line, HL-60, as well as in primary leukemic cells of the same patient, and HL-60 cells have high levels of transcripts (*32*). Gene rearrangements can also result in higher levels of c-onc transcripts. In patients with Burkitt lymphoma, c-*myc* is translocated from chromosome 8 to chromosome 14 (*33*) and there is a fivefold increase in the amount of c-*myc* mRNA compared to a control human lymphoblastoid line (*34*). Also, the translocated c-*myc* is transcribed at higher levels than the untranslocated gene in somatic cell hybrids (*35*). More recently, the c-*myc* gene product was shown to be the same size in normal and malignant cells (*36*) and these data, together with the data on differential transcription, were interpreted as an indication that an increased level of the c-*myc* protein, rather than a change in the gene product, was the relevant factor in determining transformation (*36, 37*). Translo-

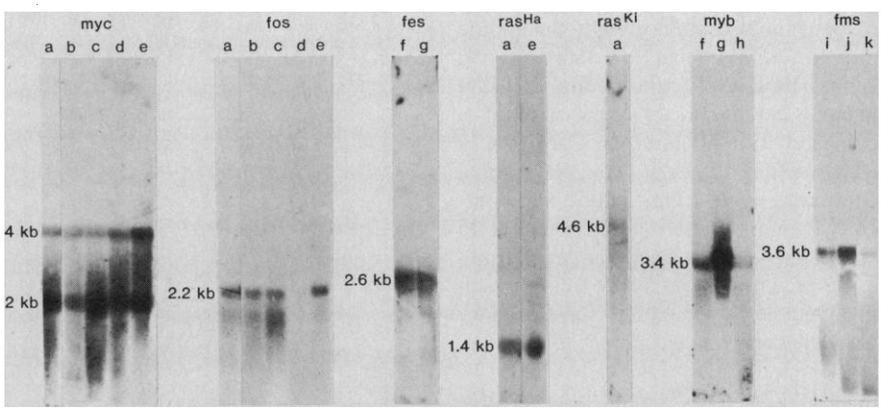


Fig. 5. Messenger RNA transcripts related to c-*myc*, c-*fos*, c-*fes*, c-*ras*^{Ha}, c-*ras*^{Ki}, c-*myb*, and c-*fms* detected in various human tumors. [Other studies have reported a c-*myb* transcript of 4.5 kb in size (*17, 23*).] Northern analysis was performed on 15 to 20 μg of poly(A$^+$) RNA as described (*22, 28*). The tumors evaluated were (a) renal cell adenocarcinoma No. 2; (b) rectal adenocarcinoma No. 1; (c) ovarian adenocarcinoma No. 2; (d) germ-cell tumor No. 1; (e) lung adenocarcinoma No. 1; (f) acute myelogenous leukemia No. 2; (g) acute myelogenous leukemia No. 4; (h) acute myelogenous leukemia No. 6; (i) breast adenocarcinoma No. 2; (j) breast adenocarcinoma No. 3; and (k) renal cell adenocarcinoma No. 8.

Table 3. Ratio of differential expression of c-onc genes in human malignant and normal tissues. The ratios of densitometry measurements of tumor to control dot blots are shown. N.D., comparison not done.

Case number	Probe				
	myc	*fos*	*ras*^{Ha}	*ras*^{Ki}	*fms*
		Renal cell carcinomas			
1	3.0	5.0	3.0	4.0	N.D.
2	4.5	4.5	3.0	3.5	N.D.
3	3.5	3.0	3.5	3.0	N.D.
5	1.0	1.0	1.0	1.0	1.0
6	1.0	1.0	1.0	1.0	1.0
8	2.0	2.0	2.0	1.0	2.5
9	2.0	1.0	1.0	1.0	6.0
		Lung carcinomas			
1	4.0	2.5	1.0	2.0	3.5
2	1.0	1.0	3.0	1.0	3.5
		Colon carcinomas			
1	11.0	3.0	3.0	4.0	N.D.
2	4.0	4.5	2.5	2.0	5.5
		Cecal carcinomas			
1	2.0	1.0	2.0	1.0	1.0
		Small bowel adenocarcinoma			
1	1.0	1.0	1.0	1.0	N.D.
		Ovarian adenocarcinoma			
1	2.5	1.0	4.0	3.0	N.D.
		Percentage of cases with differential expression			
	71	50	64	50	62

cation of the c-*abl* gene has also been described in cell line K-562, which is positive for the Philadelphia chromosome and has extremely high levels of c-*abl* transcripts (*38*). Another mechanism by which carcinogens may affect levels of c-onc expression is through altering patterns of methylation. Hypomethylation of c-*ras*^{Ha} and c-*ras*^{Ki} in malignant tissue compared to normal tissue has been reported (*39*). It is unknown, however, whether this methylation pattern correlated with different levels of expression.

The concept that an aberrant gene product may participate in the induction of malignancy is supported by the finding of a structural change in a known c-onc gene. The c-*ras*^{Ha} gene of the EJ (T24) bladder carcinoma cell line contains a point mutation at amino acid position 12 which differentiates it from its normal counterpart (*40*). This mutation apparently confers transforming potential to an otherwise normal cellular gene. However, recent analysis of 29 fresh human malignancies, including ten primary bladder carcinomas, failed to show a similar point mutation, indicating that the EJ (T24) c-*ras*^{Ha} gene is a valuable model for structural mutation of a c-onc gene but does not appear to play a role in most human epithelial malignancies in vivo (*19*). Point mutations in c-*ras*^{Ki} from human colon and lung carcinoma cell lines appear to activate this c-onc gene into a transforming gene (*41*).

The results of our experiments could be consistent with both quantitative and qualitative abnormalities of c-onc gene expression. Transcription of c-*myc*, c-*fos*, c-*ras*^{Ha}, and c-*ras*^{Ki} was higher in malignant tissue than in the corresponding normal tissue in some cases. The possibility that this may reflect heterogeneity of cell types has been discussed. If higher levels of c-onc gene expression are of pathogenetic significance in neoplasia, then this differential transcription is consistent with a quantitative model. On the other hand, the cases in which there were comparable levels of c-onc transcripts in malignant and autologous normal tissues may represent instances of structural changes in c-onc genes. Alternatively, comparable levels of c-onc gene expression in malignant and normal tissue may indicate that the genes have no role in oncogenesis in these cases. Further studies of c-onc gene structure, RNA transcripts, and protein products from fresh human tumors may help resolve these issues.

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