ONCOGENE EXPRESSION IN SOME TRANSPLANTABLE TUMORS AND CELL LINES OF RODENTS

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Since the discovery of cellular proto-oncogenes, great importance has been attributed to them because of the possible involvement of these conservative genetic elements in the processes of normal development, proliferation, and differentiation and of malignant growth. Convenient models with which to study the role of oncogenes in such important processes as tumor progression, genetic instability and heterogeneity of the tumor cell population, and metastasization are animal tumors which can be transplanted and subcultured in vitro. Despite the use of a broad spectrum of animal tumors in experimental oncology, there have so far been only isolated reports of the systematic study of oncogenes expressed in them [10]. Accordingly, the aim of the present investigation was to find tumor strains and cell lines obtained from them in which a particular oncogene is expressed stably. Tumors of various tissues obtained from rodents of different species, widely used in laboratory practice and whose biological properties have been adequately studied, were chosen for the investigation.

EXPERIMENTAL METHOD

The following strains of tumors were obtained from E. S. Revazova at the Museum of Tumor Strains, All-Union Oncologic Scientific Center, Academy of Medical Sciences of the USSR: Pliss' lymphosarcoma (LSP, rat), spontaneously metastasizing tumors of C57BL/6 mice: melanoma V-16, Lewis lung carcinoma 3LL, lung carcinoma RL-6, and Ehrlich's ascites carcinoma (EAC, mouse), and Zajdela's ascites hepatoma (ZAH, rat). The following cell lines were obtained from the above-mentioned tumor strains and used in the experiments: cell line LSK from LSP [1], cell line MM-4 from melanoma V-16, cell line LL from 3LL, cell line MAK-3 from EAC, and cell line ZAHc from ZAH. Suspension cell line KL-K5, obtained from a transplantable Svec erythromyelosis (rat) [2] and cell lines of hepatomas McA-RH 8994 and McA-RH 7777 (rat), obtained from Dr. I. E. Becker and Dr. V. R. Pottor (McArdle Laboratories on Cancer Research, Wisconsin, USA) also were used. A strain of human Ewing's sarcoma, transplantable in nude mice (ES, 32nd passage), obtained from E. S. Revazova, in which four oncogenes are expressed — les, myc, ras, and myb [12], and cell line NIH/3T3, transformed by Harvey sarcoma virus (NHS; obtained from Dr. J. Zavady, Institute of Virology, Slovak Academy of Sciences, Czechoslovakia) were used as positive controls. To isolate RNA from normal tissues, adult Wistar rats were used. Cell cultures were grown on Eagle's medium with 10% calf serum and were used in the late logarithmic phase of growth.

RNA was isolated from frozen normal tissues, cells, or peripheral areas of tumors free from necrosis, by a modified guanidine thiocyanate method, as described previously [4, 12].

Electrophoretic fractionation of RNA in 1% agarose gel with formaldehyde and hybridization of total RNA, immobilized on filters, with ^{32}P-labeled DNA from plasmids containing inserts of various oncogenes, in 50% formamide at 42°C for 48 h, was carried out as described by Maniatis and coworkers [3]. The specific radioactivity of the DNA samples used for hybridization was 2×10^8 cpm/μg DNA.

The following cloned samples of oncogenes were used: v-abl: pABlsub 3 [7], v-sis:pUCsisXP-1 (generously provided by S. L. Kolobkov, Research Institute of Biomedical Technology, Ministry of Health of the USSR); c-ras-h:pEI, 6.6 [11]; v-fos:pfos-1 [5]; v-erb-B:pAEV [13] (fragments

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TABLE 1. Expression of Oncogenes in Transplantable Tumors and Cell Lines

Strains of tumors and cell lines	Oncogenes							
	myc	fos	ras	abl	erb-B	Blym	sis	myb
LSP	2,5	—	±	—	—	—	—	—
LSK	2,5	3,5 2,2	±	—	—	—	—	—
KL-K5	2,5	3,5 2,2	±	—	—	—	—	—
3LL	2,5	+	±	—	—	—	—	—
LL	2,5	+	±	nt	nt	nt	—	—
V-16	—	+	±	—	—	—	—	—
MM-4	—	+	±	—	—	—	—	—
EAC	2,5	±2,2	±	—	—	—	—	—
MAK-3	2,5	+2,2	±	—	—	—	—	—
RL-6	2,5	+	±	—	—	—	—	—
ZAH	—	—	+	—	nt	—	—	—
ZAHc	—	—	+	—	nt	nt	—	—
McA-RH 7777	—	—	5,4 1,2	—	nt	nt	—	—
McA-RH 8994	—	—	+	—	nt	nt	—	—
NIH-NHS	2,5	2,2	6,4 4,8 2,7 2,0	—	nt	—	—	—
ES	+, 2,5	+, 2,2	+, 5,4	—	—	+, 2,4	—	4,0

Legend. Numbers indicate size of RNA, in kbp; —) absence of expression, results read only after control hybridization, revealing presence of mRNA of actin in total RNA preparation; +) diffuse hybridization band; nt) not tested.

of oncogene erb-B, expressed from the plasmid at the Bam H1 site were used); c-Blym-h: pHuBlym-1 [6]; v-myc:puMyC3 [14]; c-myb-h:pKH47, 2.8 [8].

EXPERIMENTAL RESULTS

To analyze expression of mRNA specific for oncogenes by the Northern blotting method, total RNA preparations were used (30 μg for each lane on the gel) and hybridization was carried out with plasmids containing fragments of DNA of the oncogenes. The sensitivity of the method is such that 15-20 copies of specific RNA per cell can be determined. The level of oncogene expression in normal tissues is below this value [4, 12].

As the results in Table 1 show, expression of various oncogenes was found in all the tumors and cell lines tested, and its level was higher than that in normal tissues (liver, thymus, spleen). The spectrum of expressed oncogenes, the level of their transcription, and the size of the transcripts were specific for each strain, and are evidently independent of the tissue or species of the tumor. For instance, simultaneous expression of oncogenes myc and fos was observed in cells RL-6, 3LL, and EAC (mammary gland), human ES (bone tissue), and cell lines of leukemias LSK and KL-K5.

The oncogenes used could be divided according to the character of their transcription into two groups: frequently expressed and weakly expressed or "silent." The number of frequently expressed oncogenes includes ras, myc, and fos, transcription of which was found in more than 50% of samples tested. In the case of the ras oncogene, discrete hybridization bands with transcripts of 5.4 and 1.2 kbp, the usual size for this family, were observed for only one of all the RNA samples tested (McA-RH 7777); in the remaining cases weak diffuse hybridization bands were recorded. Expression of oncogenes such as myb, abl, sis, erb-B, and B-lym could not be detected in any of the rodent tumors studied. Increased expression of B-lym with an unusual size of transcript (2.4 kbp) was found only in the human ES, in which, besides the four oncogenes hitherto known, expression of two more could be detected: B-lym and fos. A similar picture of distribution of oncogenes among two groups was observed previously in other rodent tumors and cell cultures [10], and also during a study of primary human tumors [4, 12].

It is important to note that the set of expressed oncogenes characteristic of the tumors from which they were obtained was preserved in the cell lines. This phenomenon was observed for melanoma V-16 and line MM-4, EAC and line MAK-3, ZAH and line ZAHc, and 3LL and line LL. The only exception was line LSK, in the cells of which, unlike the original tumor of strain LSP, expression of the fos oncogene appeared (2.2 and 3.5 kbp). This feature of the LSK line can be explained on the grounds that in the logarithmic phase of growth (doubling time 12 h)

the percentage of cells in the early G_1 phase of the cell cycle, in which a physiological "flash" of expression of the fos oncogene is observed [9], was significantly higher in cell line LSK than in the original tumor. For some strains and lines (ZAH and ZAHc, EAC and MAK-3, McA-RH 7777 and 8994) the investigations were conducted on different passages, but under these circumstances no differences were found in the set of oncogenes expressed, the level of expression, or the size of the transcripts. It can be postulated on the basis of the foregoing remarks that oncogene expression in transplantable tumors may be a strain-specific and sufficiently conservative feature, preserved on culture of these cells in vitro. This feature can probably be used for categorization of the strains.

Thus tissue-specific expression of oncogenes was not found in the rodent tumors investigated, just as was the case also in human tumors. It was shown that stable expression of oncogenes is possible in conversion of tumors transplantable into animals into cell lines and during long-term passage of these cell lines in vitro. The character of expression of oncogenes and, in particular, myc, fos, and ras, likewise is unconnected with the ability of the tumor cell strains to give spontaneous metastases.

At least six tumor strains and cell lines were thus found (LSK, KL-K5, 3LL and LL, MAK-3 and EAC, RL-6, and McA-RH 7777) which may be used as models with which to study the biological role of oncogenes such as myc, fos, and ras.

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